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PLASTIC STUDIES IN ABNORMAL RENAL ARCHITECTURE

VI. AN INVESTIGATION OF THE CIRCULATION IN INFARCTS OF THE KIDNEY

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The work presented here was started originally as an investigation of infarction in human kidneys by the methods which Oliver and co-workers [1] used in their plastic studies of abnormal renal structure. In the course of the work certain questions arose which made it appear advisable to use the experimentally infarcted kidneys of animals as well. The advantages of studying experimentally produced infarcts coincidentally with those in human kidneys are that the time and place of the experimental interruption of the circulation are known, and complicating factors, which may be present in the human subjects, such as septic emboli and arteriosclerotic lesions, are absent. Also the whole kidney of a small animal can be viewed under the dissecting microscope, and the parts dissected can be kept in relation to the whole or any other part. The similarity of the structures in the human and the animal kidney has been sufficiently demonstrated by extensive research so that it is possible, with some reservations which will be mentioned, to draw conclusions about one from the other as to the lesions produced by simple ischemia.

The material for this paper is therefore chiefly derived from the infarcted kidneys of rats and deals particularly with the circulation in

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1. (*a*) Oliver, J., and Lund, E. M.: Arch. Path. **15**:755, 1933. (*b*) Oliver, J., and Luey, A. S.: ibid. **18**:777, 1934; **19**:1, 1935. (*c*) Loomis, D.: ibid. **22**:435, 1936. (*d*) Strong, K. C.: ibid. **29**:77, 1940.

the injured areas. A later communication will present our findings on parenchymal alterations.

Based principally on the work and views of Rokitansky,[2] Cohnheim,[3] Foà,[4] Ribbert[5] and Karsner and Austin,[6] the following conception of the results of obstruction of a branch of the renal artery is commonly held and presented in textbooks. Following immediately on the obstruction there is dilatation of the capillaries at the periphery of the affected area. An influx of blood through these vessels occurs to a varying extent into the vascular bed supplied by the obstructed branch of the artery from those supplied by the unobstructed branches of the artery. The ischemic damage to the capillaries results in hemorrhage and edema, and the final stasis is followed by conglutination of the erythrocytes and decolorization of the hemoglobin. Necrosis of the parenchyma, except for a marginal zone, which is called the zone of reaction, follows. In this zone of reaction there is hyperemia, and later, a certain amount of replacement of the tubular epithelium by an atypical epithelium. A proliferation of capillary endothelium and fibroblasts takes place at the periphery of the infarct, and new vessels and fibroblasts invade it and replace the necrotic tissue with granulation tissue. The end result may be a poorly vascularized scar or, if the infarct is large, a persistent necrotic mass, sometimes containing calcium, surrounded by connective tissue.

The foregoing commonly accepted teaching is the result of gross and histologic study of naturally occurring infarcts in human kidneys and infarcts experimentally produced in animals. Other methods of investigation have been used by Litten,[7] Bier,[8] Forbes[9] and Karsner,[6] who attempted to identify the source of the collateral circulation and to study the effects on the infarct of various manipulations, such as ligating ureteral and capsular arteries, decapsulation, ligating the ureter and ligating the companion vein as well as the artery. Ribbert[5] and Gänsslen[10] studied circulatory conditions by injecting a colored mass

2. von Rokitansky, C.: Handbuch der pathologischen Anatomie, Vienna, Braumüller & Seidel, 1844, vol. 2.

3. Cohnheim, J.: Untersuchungen über embolischen Processe, Berlin, A. Hirschwald, 1872.

4. Foà, P.: Beitr. z. path. Anat. u. z. allg. Path. **5**:275, 1889.

5. Ribbert, H.: Virchows Arch. f. path. Anat. **155**:201, 1899.

6. Karsner, H. T., and Austin, J. H.: J. A. M. A. **57**:951, 1911.

7. Litten, M.: Ztschr. f. klin. Med. **1**:131, 1880.

8. Bier, A.: Virchows Arch. f. path. Anat. **153**:306, 1898.

9. Forbes, D.: A Study of Experimental Infarcts of the Kidney, Thesis, Edinburgh, 1901; cited by Beattie and Dickson.[41]

10. Gänsslen, M.: Ergebn. d. inn. Med. u. Kinderh. **47**:275, 1934.

into the arterial system of the infarcted human kidney, and the same procedure was followed by MacNider [11] with the cat's kidney. Belt and Joelson [12] by the injection and corrosion of infarcted kidneys of dogs showed anastomoses which developed after the severing of a large branch of the renal artery.

The lesion in the kidney produced by cutting off the circulation has, however, never been elucidated by the method of dissection until now. By perfusion and dissection we have been able to discover what circulatory channels are put into use as a result of obstruction, how quickly and effectively the collateral circulation acts and exactly where and to what extent tissue changes occur.

A brief outline of the circulation of the kidney seems to us valuable for reference as well as for placing in perspective divergencies of opinion which have caused confusion in the past.

THE DIRECT BLOOD SUPPLY OF THE RENAL PARENCHYMA

All the blood which passes into the intertubular network of the kidney does so by way of the efferent arterioles after traversing the glomerular capillaries. There are the following minor exceptional paths:

1. Ludwig's branches, which are minute and very rare except as the circulation of the kidney evolves with aging and in conditions of disease.

2. Direct arteriolar branches from the interlobular arteries. These also are very few, but the number becomes increasingly great with age and in disease.

3. Arteriolae rectae verae. These are a special case of 2 and are present in significant numbers only under the same conditions, although the older anatomists and some pathologists (Virchow [13] and MacNider [11]) dignified them as a separate category of vessels and considered that they supplied a large proportion of the medullary tissue.

These statements are supported by the work of Bowman,[14] Huber,[15] Gänsslen,[16] Hou-Jensen,[16] von Möllendorf,[17] Oliver,[1] Loomis [18] and MacCallum [19] and are further confirmed and amplified by recent investi-

11. MacNider, W. de B.: J. M. Research **24**:425, 1911.

12. Belt, A. E., and Joelson, J. J.: Arch. Surg. **10**:117, 1925.

13. Virchow, R.: Virchows Arch. f. path. Anat. **12**:310, 1857.

14. Bowman, F. R. S.: Phil. Tr. Roy. Soc. London **132**:57, 1842.

15. Huber, G. C.: Am. J. Anat. **6**:391, 1907.

16. Hou-Jensen: Ztschr. f. Anat. u. Entwcklngsgesch. **91**:1, 1929.

17. von Möllendorf, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 7, pt. 1.

18. Loomis, D.: Arch. Path. **22**:435, 1936.

19. MacCallum, D. B.: Am. J. Anat. **38**:153, 1926.

gations of our own. A definitive answer to the long disputed question as to whether the kidney "normally" has aglomerular arterial branches or not has been made by Oliver [20] as follows:

> To return to the problem as to whether the normal vascular bed of the kidney includes direct branches, the solution of a long continued controversy is found in the realization that the normal life history of the arteries includes the development of sclerotic changes. . . . Oppenheimer has shown that they begin early in life and that they are found constantly in later years. The conclusion is obvious, therefore, that the adaptive and probably compensatory development of new vessels (an accompaniment of this sclerosis) must also be considered a part of the life history of the renal arteries and characteristic of the structure of the senile kidney. Vasa rectae verae, therefore, (and other direct branches) are not normally found in significant numbers in the kidney of the young adult though in the kidney of 70 years they are "normal" constituents of the arterial tree.

THE ARTERIAL ANASTOMOTIC SUPPLY

Communications Between Extrarenal and Intrarenal Vessels.—(*a*) Between Arcuate and Extrarenal Arteries: In the human kidney one, and rarely more than one, aglomerular perforating artery may arise from an arcuate artery and anastomose with perirenal arteries. This is not found in all human kidneys (Brödel [21]; Gänsslen [10]) and has not been described in other mammalian kidneys.

(*b*) Between Interlobular and Capsular Arteries: Terminal divisions of interlobular arteries are believed by many to pass directly to the capsule and to anastomose there with capsular vessels. Dehoff [22] found them to be numerous, but much doubt has been thrown on the correctness of her findings by later workers. Belt and Joelson [12] found vessels of this type in the normal dog's kidney. Huber [15] stated them to be more common in the dog than in other animals but to occur even in that species only occasionally. Langley [23] and Morison [24] have also described them as few. Gänsslen [10] found them to be rare in the human kidney. We have found only one example after exhaustive search of many rat kidneys, though these vessels may appear in greater number in disease, as the findings in this paper will show.

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20. Oliver, J.: Urinary System, in Cowdry, E. V.: Problems of Ageing, Baltimore, Williams & Wilkins Company, 1939, chap. 10, p. 270.

21. Brödel, M., in Kelly, H. A., and Burnham, C. F.: The Diseases of the Kidneys, Ureters and Bladder, New York, D. Appleton and Company, 1914, vol. 1.

22. Dehoff, E.: Virchows Arch. f. path. Anat. **228**:134, 1920.

23. Langley, J. N.: J. Physiol. **60**:411, 1925.

24. Morison, D. M.: Am. J. Anat. **37**:53, 1926.

(*c*) Between Branches Exterior to the Kidney: Communications exist between vessels of precapillary and capillary size, not only between branches of the renal artery but also between branches of the renal artery and those of neighboring arterial systems, such as the lumbar, the ureteral and the ovarian, in the perirenal fat, the capsule, the pelvis and the ureter (fig. 1). The renal artery just as it enters the renal substance supplies the pelvis with a few short branches which have both glomerulus-bearing and non-glomerulus-bearing twigs, and these anastomose with the other pelvic vessels by capillary connections (fig. 1). In the rat the most interior part of the pelvis is supplied by the efferent arterioles from nearby glomeruli. Hou-Jensen [16] stated that the pelvis in the human kidney is also supplied by direct branches from the arcuate arteries.

Intrarenal Arterial Communications.—These are composed chiefly of the divisions of the efferent arterioles, are of precapillary and capillary size and are part of the intertubular network (fig. 1, normal area; figs. 15 and 16). They are particularly easy to observe on the surface of the kidney (figs. 27 and 28*a*). They are entirely separated from the capsular vessels in the rat's kidney. They constitute the only communication between arterial branches when obstruction to circulation occurs beyond the point where the recurrent branches leave the renal artery to supply the ureter, pelvis and capsule. Hou-Jensen [16] found that in the human kidney aglomerular branches from the arcuate arteries supply the adventitia of the larger vessels. There is a possibility that anastomoses between such branches can thus connect one arcuate artery with another. These were not observed by us in the rat.

In addition to the anastomotic connections enumerated in the foregoing paragraph, others have been described which are mentioned separately because of the lack of corroboration of their existence by the many careful observers in the field and our own failure to find them after diligent search. The disorder which their presence would create in an otherwise dynamically balanced system should make it seem unlikely a priori that such arrangements exist. We refer to the relatively large and numerous arteriovenous anastomoses reported as existing between interlobular arteries and veins by Steinach,[25] Golubew [26] and Spanner.[27] Hou-Jensen [16] examined this point carefully and was unable to find such anastomoses in human kidneys. Von Möllendorf [28] also appears to have

25. Steinach, E.: Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. cl. **90**: 3 and 171, 1884.

26. Golubew, W. Z.: Internat. Monatschr. f. Anat. u. Physiol. **10**:541, 1898.

27. Spanner, R.: Verhandl. d. anat. Gesellsch. **45**:81, 1938.

28. von Möllendorf,[17] pp. 116-117.

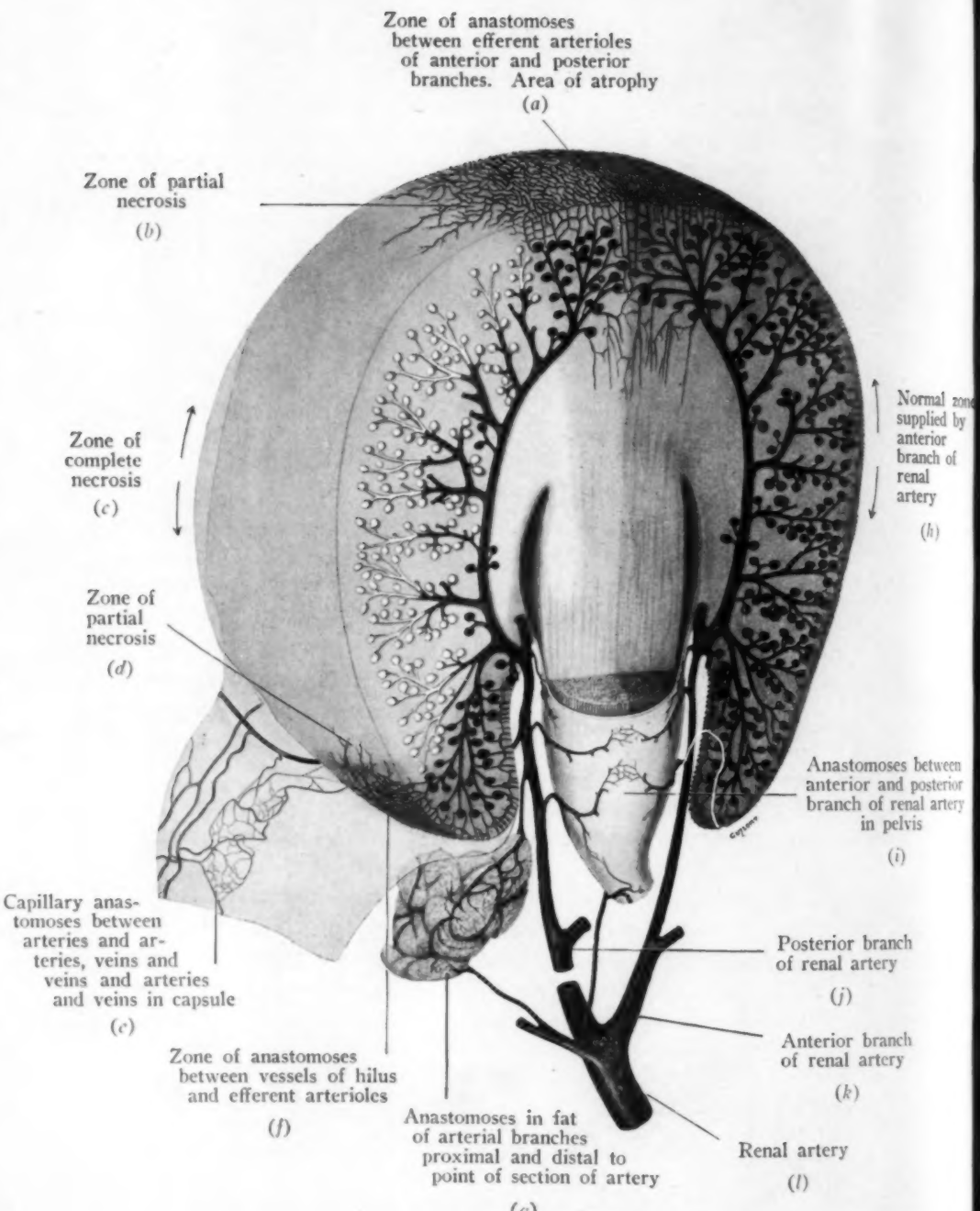

Figure 1

(See legend on opposite page)

been dubious as to their existence. In our dissections of over a hundred kidneys we have never found a single example.

If we consider only those vascular elements the existence of which has been confirmed by the work of many careful investigators as normal to the circulation of the kidney and disregard arteriovenous shunts as not proved to occur, we are presented with a dynamically perfect system for the distribution of blood. Each final unit, the glomerulus, is in equilibrium with its neighbor. Although each glomerulus is connected through the intertubular capillaries on all sides with neighboring glomeruli, ordinarily blood from one glomerulus cannot pass to another or into the network beyond a specific zone of action because it meets another current of blood of opposing and equal force. Thus it can follow only the course of diminishing pressure through the capillaries to the veins. The pressure relation or balance in these practically identical structures through which operate simultaneously equal forces makes possible a continuously uniform and systematic distribution of blood to the kidney tissue.

When these pressure relations are altered by obstruction of a vessel and the barrier of an opposing force is removed, the blood from the glomeruli of an unobstructed vessel will flow as far through the capillary network of the obstructed zone as the vis a tergo will permit. This vis a tergo will be lessened by the constant draining off of a certain portion of the blood by the veins, and resistance to it will be offered by the inert column of fluid already present in the vessels of the obstructed zone and by the increasing viscosity as fluid escapes through the capillary walls. There will be many points where effective movement is impossible, with the result that selective channelizing occurs in those paths in which the blood can flow freely and continuously. If the arteries in the neighboring region supplied by an unoccluded branch are able to dilate sufficiently, they can conduct an increased volume of blood to their finest branches and into the capillary communications with the occluded artery proportional to the amount of blood that the occluded vessel formerly carried.

EXPLANATION OF FIGURE 1

Semidiagrammatic composite representation of the arterial circulation of an infarcted rat kidney assembled from drawings and photographs of undissected and dissected material. This represents the condition in the infarct about one week after infarction.

In the experiments which we shall now describe one may see how these factors acting together in an infarcted area produce the alterations that are observed in the disturbed vascular bed.

EXPERIMENTAL METHODS

Operation.—The left kidney of a rat under ether anesthesia was exposed by a lumbar incision with aseptic technic. As an especial precaution against the kidney's being damaged by drying or handling, it was inserted with the aid of eyelid retractors through a slit in a sterile square of silk soaked in warm sterile saline solution and was held by the corners of the cloth away from the field of operation. It was necessary to remove some of the fat attached to the capsule at the poles, but the kidney itself was never grasped with the fingers or with instruments, and the capsule was rarely damaged. The renal artery, which divides into two approximately equal branches a little distance from the hilus, was isolated from the vein with the blunt end of a darning needle which had been slightly curved in a flame and mounted in the handle of a dissection needle. Ligatures of silk were placed around the posterior branch of the artery, which was then cut between the ligatures. In some cases the artery was ligated but not severed. Before the wound was closed, about 3 cc. of warm sterile saline solution was introduced into the cavity. The edges of the abdominal wall and the skin were sutured separately with silk.

Perfusion.—When the desired time after operation, one minute to one year, had elapsed, the animal was anesthetized, and the peritoneal and the thoracic cavity were opened, a cannula introduced into the aorta at the level of the diaphragm and the animal perfused with warm physiologic solution of sodium chloride followed by Higgins' engrossers ink in a dilution of 1 part ink to 8 parts of warm saline solution (Winternitz [29]). The introduction of the saline solution into the aorta began in most cases before the heart stopped beating. About 1,000 cc. of saline solution was perfused over a period of about one-half hour with a pulsating pressure (Winternitz [29]) ranging between 100 and 200 mm. of mercury. The pulsations were approximately 60 per minute and of an amplitude of 50 mm. of mercury. The normal systolic pressure of the rat is 106 to 145 mm. of mercury (Byrom and Wilson [30]). This can rise as high as 260 mm. of mercury as a result of compression of the renal artery (Wilson and Byrom [31]). The saline solution was followed by 1,500 to 2,000 cc. of the diluted ink perfused at the same pressure over a period of one to two hours.

The kidney was removed after perfusion and fixed in 10 per cent solution of formaldehyde U. S. P. Usually it was cut in half across the middle of the infarct, one half being used for dissection and the other half for histologic sections. Occasionally the whole kidney was used for dissection. Several kidneys were perfused by way of the renal vein with a steady pressure of 80 mm. of mercury.

It can be seen that a great deal of pains was taken to approximate as nearly as possible the physiologic conditions in the living animal by

29. Winternitz, M. C.; Thomas, R. M., and LeCompte, P. M.: The Biology of Arteriosclerosis, Springfield, Ill., Charles C. Thomas, Publisher, 1938.

30. Byrom, F. B., and Wilson, C.: J. Physiol. **93**:301, 1938.

31. Wilson, C., and Byrom, F. B.: Lancet **1**:136, 1939.

our methods of perfusion, in contrast with the usual practice of injecting postmortem material which has been removed from the body. We have found india ink more satisfactory for fine detail than celloidin (a preparation of pyroxylin) dissolved in acetone, which has been used by some workers, although in Huber's [15] hands this method gave very excellent results. Zweifach [32] showed the value of particulate matter in the perfusion fluid, especially for injection of capillaries, and Kurkowsky [33a] emphasized the importance of perfusion of the animal's vascular bed in situ before postmortem change has set in. It is possible that the use of postmortem tissues by the earlier workers accounts for the errors in their descriptions of the structure of the renal vascular system, because complete injection of the entire vascular bed is accomplished at best with difficulty. Huber came to the conclusion that it was futile to attempt fine injection of human kidneys or of kidneys of animals dead more than a few hours.

Dissection.—The fixed material was macerated in concentrated hydrochloric acid until soft enough for dissection. The period of maceration depended on the temperature of the room and varied between eighteen and forty-eight hours. After being washed thoroughly, the tissue was dissected in water under a binocular microscope, and stereoscopic photographs and drawings were made of the preparation. Uninjected specimens of both rat and human kidneys were also studied in dissection.

OBSERVATIONS

With such a technic, the presence of india ink in blood vessels demonstrates that the lumens of these vessels are patent and connected in some manner to the source of the perfusion fluid. Lack of ink is evidence either that the lumens are obstructed or that sufficient fluid has not been able to penetrate through devious and narrow paths to reach them. As it is certain that the blood flow working continuously under physiologic conditions is able to penetrate more efficiently and therefore farther than perfusion fluid flowing under similar pressure but for a short period, we feel justified in making the assumption that blood was flowing during life to the same points where india ink is found in the vessels. A comparison of perfused material with histologic sections of unperfused kidneys showing discrete red blood cells in vessels and glomeruli in the infarcted tissue supports this assumption (figs. 2, 3, 4, 5, 6 and 7).

A few minutes to four hours after the posterior branch of the left renal artery is severed or ligated, the affected kidney, perfused by way

32. Zweifach, B. W.: Proc. Soc. Exper. Biol. & Med. **44**:124, 1940.

33. Kurkowsky, W.: (*a*) Ztschr. f. Anat. u. Entwcklngsgesch. **98**:126, 1932; (*b*) **105**:108, 1935.

of the aorta, has small quantities of india ink in those intertubular capillaries of the involved area which anastomose directly with the capillaries springing from efferent arterioles of the glomeruli that are borne on branches of the nonligated anterior division of the renal artery. Ink is also present in some of the veins. A very minute amount of ink is seen in the lumen of the obstructed posterior branch of the artery a little distal to the point of interruption.

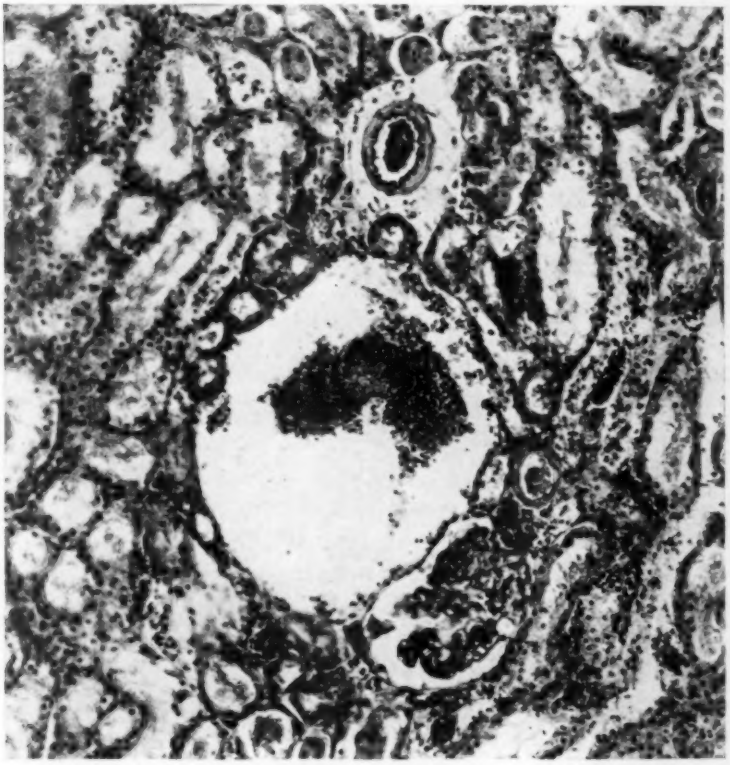

Fig. 2.—Artery, vein and glomerulus near the corticomedullary junction well within the infarcted area twenty-four hours after infarction. Note the thrombi in the artery and the vein, as well as the discrete erythrocytes. The glomerulus is well preserved. Its capillaries also show discrete red blood cells, Edema is seen around the artery. Rat C-54; × 150.

In the specimen prepared *twenty-four hours* after the interruption of the circulation, the amount of india ink found in the large divisions of the posterior renal artery is increased, while the glomeruli on its branches nearest the anterior half of the kidney show a speckling of india ink in the tufts and afferent arterioles (fig. 9). The segment of

the artery distal to the obstruction where recurrent branches go to the ureter, pelvis, capsule and fat (fig. 1 *g, i*) contains the most india ink, and some of the glomeruli here are completely injected.

Fig. 3.—Vessels from the center of the infarct near the corticomedullary junction three days after infarction. The glomerulus is fully preserved. Discrete erythrocytes are present in the vein. Red blood cells and edema are seen in the arterial wall. There is beginning regeneration of tubular epithelium. Rat C-76; × 300.

Three days after the production of the infarct (fig. 8) the india ink penetrates into more glomeruli in the same area as described in the foregoing paragraph, but instead of showing scattered particles, the tufts are now well injected. In the more central portions of the infarct, where the vessels have previously shown no ink, this material is now found to have entered arcuate arteries and even some interlobular arteries from the larger divisions of the artery. That the ink has

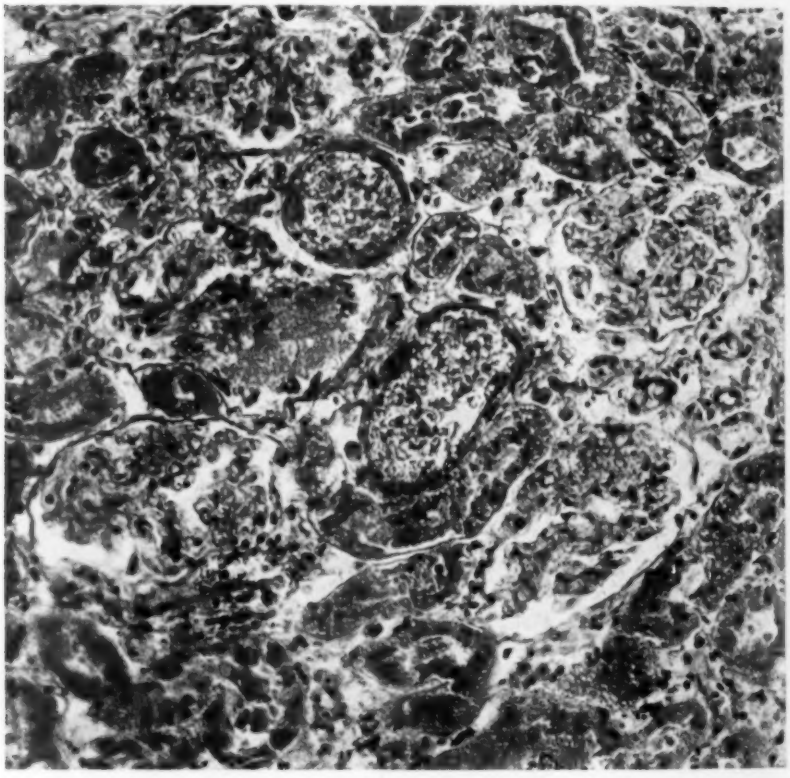

Fig. 4.—One week after infarction the interlobular artery contains fresh erythrocytes. The edema in the adventitia has diminished. The nuclei in the neighboring glomeruli show pyknosis, but the capillaries contain discrete red blood cells. Rat C-88; × 300.

entered from the larger branches and not through the glomeruli of this region is shown by the fact that the upper parts of these branches and their most distal glomeruli do not show the presence of perfusion fluid. On the other hand, those glomeruli near the corticomedullary junction which are very close to the large main branches of the artery are well injected (figs. 1 and 8).

After one week (fig. 1) the injection fluid in the majority of the arterial branches still rises only part way, and most of the distal parts of the interlobular arteries and all of the afferent vessels and glomeruli in the center of the infarct still remain empty. Thrombi in many of

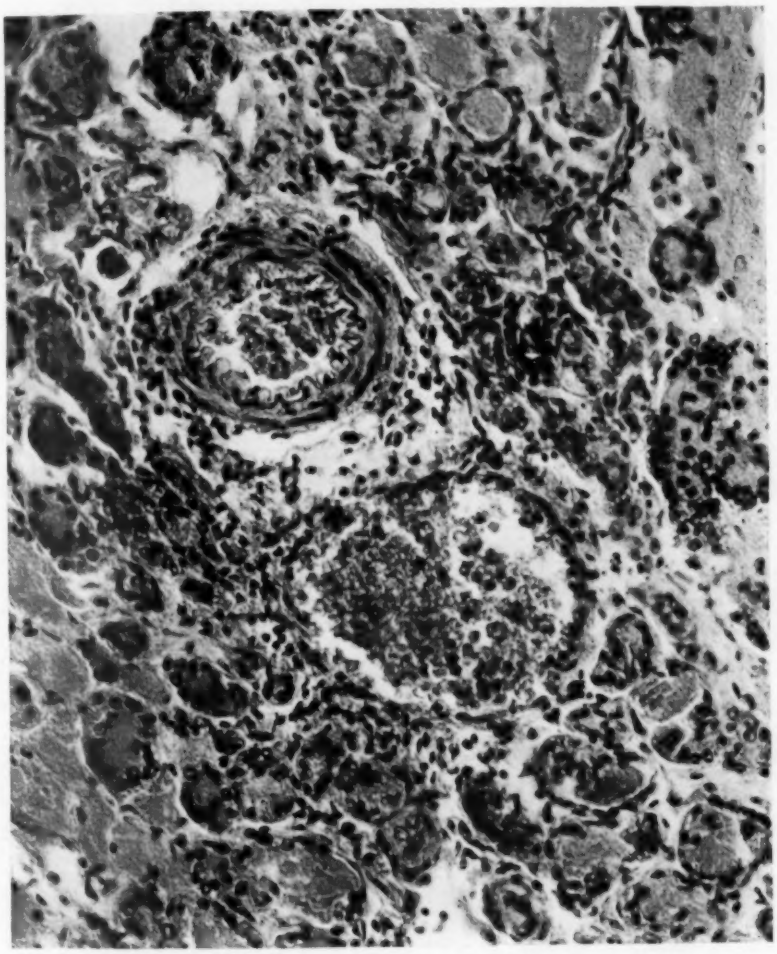

Fig. 5.—Two weeks after infarction the vein and the artery near the corticomedullary junction in the center of the infarct contain blood. The wall of the artery is thickened, and there is proliferation of connective tissue in the adventitia. There has been regeneration of an atypical epithelium in the neighboring tubules. Rat C-67; × 300.

these vessels partially or completely block the flow of fluid (fig. 10). These vessels also show contractions and dilatations, and in many the lumen appears as a thin line.

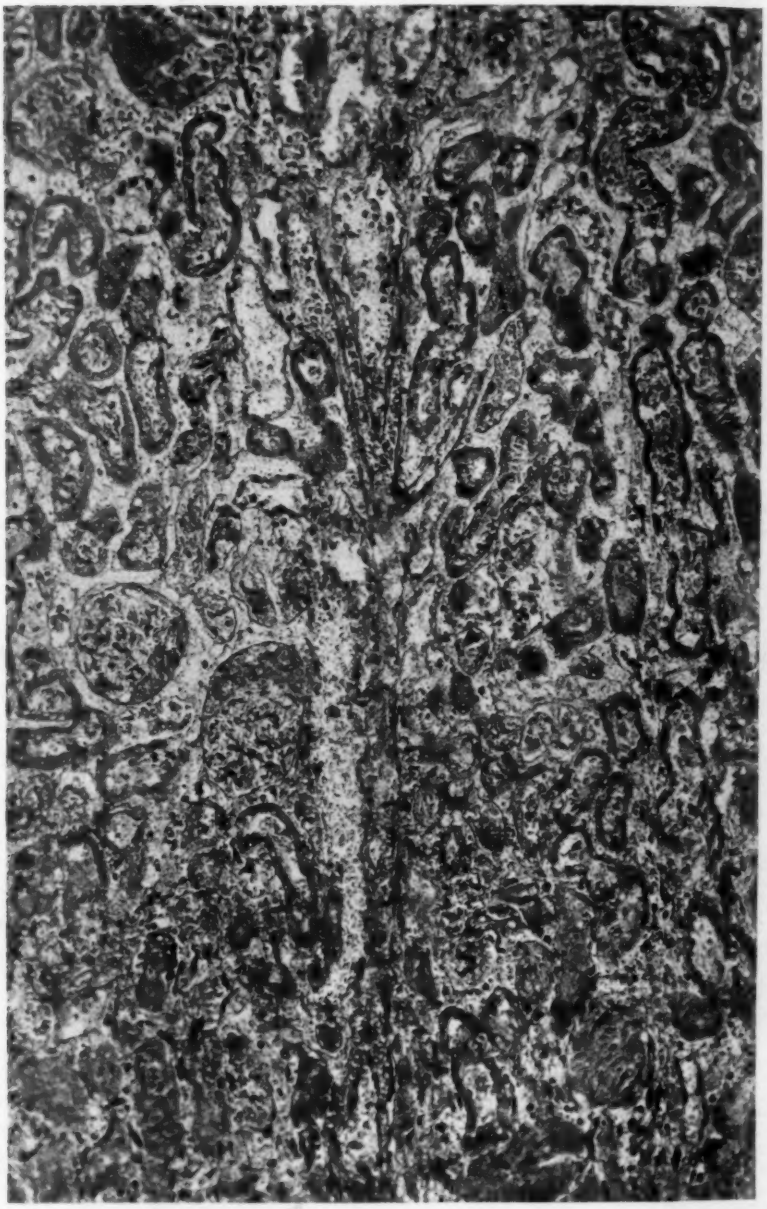

Fig. 6.—Six days after infarction the interlobular artery and vein in the center of the infarct show discrete erythrocytes in the lower segments and debris in the upper segments with necrosis of the walls. Rat C-98; × 150.

Fig. 7.—A thrombus and well preserved red blood cells are seen in the lumen of an interlobular artery near the center of the infarct one week after infarction. The endothelial lining is regenerating. There is fibroblastic proliferation in and around the vessel. Rat C-34; × 150.

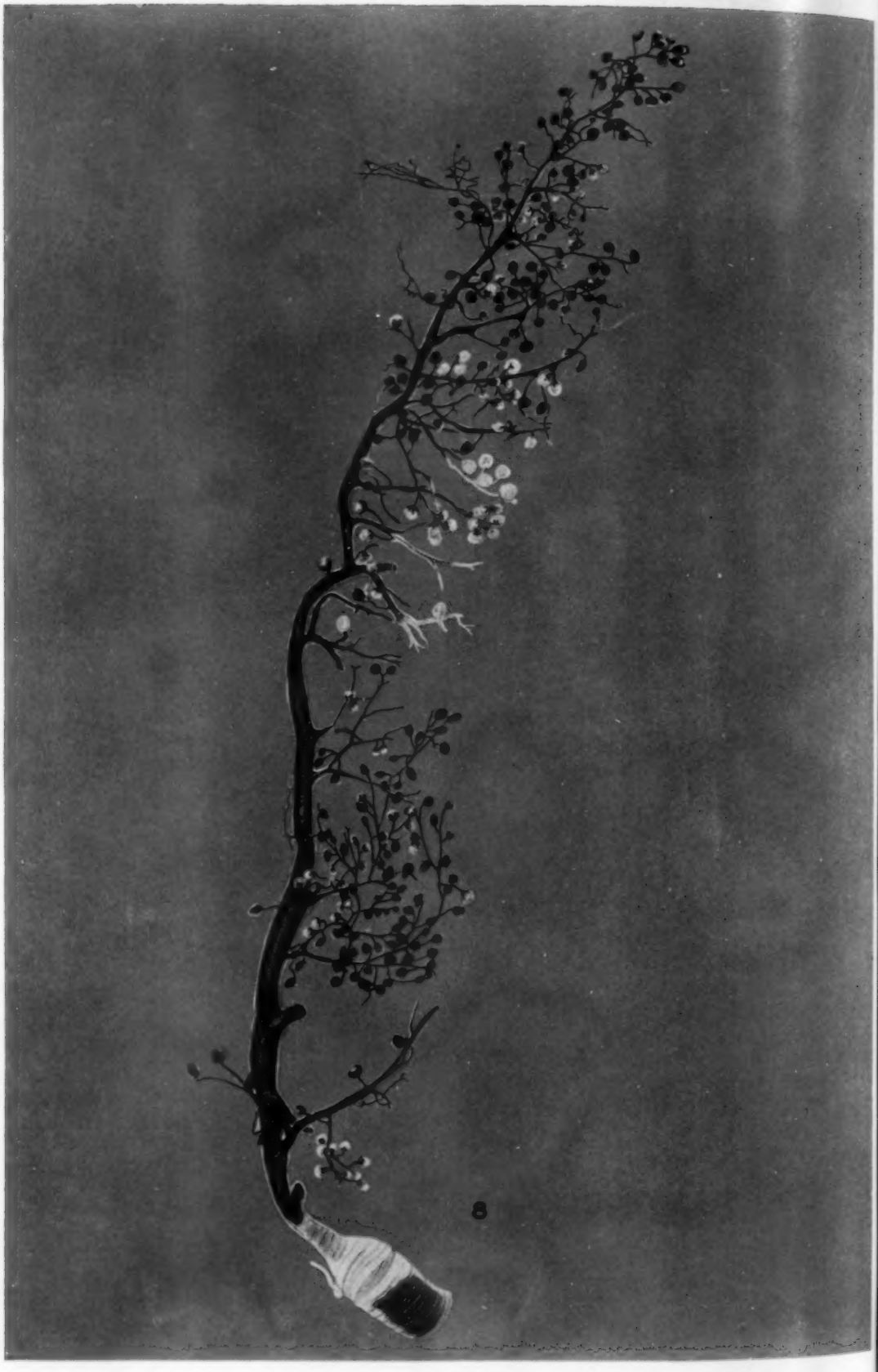

Figure 8

(See legend on opposite page)

A histologic examination of the conditions of these arteries at an earlier date (figs. 2, 3 and 6) reveals edema in the adventitia and infiltration of erythrocytes into the arterial walls. At one week the lesion has progressed to fibrin deposits, hemoglobin droplets, hemosiderin and nuclear debris in the lumens of such vessels, and necrosis of their walls is observed (figs. 6 and 7).

The passage of injection fluid through the capillary network to the interlobular veins throughout the infarct is evident at this time. The veins in the center of the infarct have lost the normal bushy appearance which is ordinarily given them by their many tributaries and are thin and sparse. The only veins in the infarcted area which retain their bushy appearance are those nearest the tissue supplied by the anterior branch of the renal artery, and there it is even accentuated beyond normal because of the dilatation of the venules.

The capillary system in the main part of the infarcted tissue now shows some degree of filling, but the complex appearance that it presents in normal tissue has changed to a simplified form. One or two paths appear to have been chosen out of the many which previously existed, and these run a fairly straight course accompanying the collecting tubules from the surface of the cortex to the corticomedullary junction, where they enter the venous arcade. In the intermediate zone of necrotic tissue some filled branches angle off from these capillary pathways and approach the deeper glomeruli by way of the efferent arterioles (fig. 29).

The injection fluid also flows through the more superficial capillary network on the surface of the cortex, where a new vascular architecture is seen evolving from the old. Some of the capillaries are enlarged (figs. 27 and 28) until they become fair-sized vessels, while others cannot be visualized with india ink and are undoubtedly lost to the system. Instead of the usual small-meshed capillary network of almost uniform vessels in great abundance, branching and uniting in every direction around the tubules, a simplified network consequently arises, infrequently connected and composed of vessels of varying caliber.

EXPLANATION OF FIGURE 8

Dissected specimen of a large division of the posterior branch of the renal artery three days after ligation. The point of ligation is marked by a thrombus and a reduction in the size of the artery. The ink has penetrated more completely into the glomeruli nearest the hilus and into the most distal part which borders on the zone of the area supplied by the unligated anterior branch. The intermediate zone is less fully injected. Many of the branches in this area were lost in dissection because of the difficulty of following the unfilled channels to their termination. Rat C-107; × 15.

Figures 9 to 14

(See legends on opposite page)

The larger channels are clearly seen to originate from and to be dispersed into much smaller units (fig. 28) and are not outgrowths from large arterial branches. They represent a remodeling of the original capillary channels, which have become enlarged by their use as main pathways in the new conditions of pressure imbalance caused by the obstruction. These changes will be discussed later.

The great dilatation of capillaries, venules and veins within the infarcted area close to the zone supplied by the unobstructed anterior branch is observable from the first and continues to develop until the vessels are so conspicuous and distended that they appear to form a veritable thicket at the periphery of the infarct. They surround the interlobular arteries in a dense mass and must be broken away to reveal the more delicately injected artery and glomeruli. The hedgelike appearance is caused by the coincident atrophy of the parenchyma and the abundant filling of the dilated vessels.

This dilatation is permanent and affords easier passage for the perfusion fluid and presumably, therefore, for blood than those vascular

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EXPLANATION OF FIGURES 9 TO 14

Fig. 9.—A terminal branch of the ligated artery from the zone of capillary anastomosis with the anterior branch system. This shows penetration of india ink twenty-four hours after infarction into the most distal glomeruli and branches, from the zone supplied by the anterior unligated branch of the renal artery. Rat C-60; × 30.

Fig. 10.—One week after infarction the india ink has penetrated part way into the interlobular arteries of the central part of the infarct. The glomeruli remain uninjected. Thrombi are seen sometimes completely but usually only partially blocking the flow of ink. Rat C-131; × 30.

Fig. 11.—Interlobular artery at one week after infarction from the zone of complete necrosis of the parenchyma. The injection fluid is blocked part way in its course to the periphery. Rat C-131; × 30.

Fig. 12.—Interlobular artery two weeks after infarction from the same area as is shown in figure 11. Shrinkage of the artery and of the glomeruli has occurred. There has been an extension of the patent lumen in a thin-walled vessel which unites the interlobular artery with the superficial capillary network. Rat C-73; × 30.

Fig. 13.—A distal division of the ligated artery injected two weeks after infarction. The glomeruli nearest the anterior zone (*a*) are well injected, though somewhat reduced in size; the branches which show uninjected shrunken glomeruli and the interlobular arteries very much reduced in diameter are directed toward the more interior parts of the infarct. Rat C-73; × 30.

Fig. 14.—A dissection of an injected interlobular artery two weeks after infarction from the same area as figure 13 (right) compared with a normal interlobular artery (left) to show the diminished size of the glomeruli and artery. Rat C-73; × 30.

Figures 15 to 26

(See legends on opposite page)

pathways presented by the undisturbed capillaries and venules directed away from the infarcted area into the normal tissue, since the veins at the periphery of the infarct are found to be well filled even in specimens in which the veins of the uninfarcted area contain little india ink.

Explanation of Figures 15 to 26

Fig. 15.—A normal interlobular artery with efferent arterioles and part of the intertubular network showing anastomoses between glomeruli both in the cortex and in the medulla. The glomerulus (→) that should be at the lower right, shown by dotted line, is broken off, but part of its efferent vessel is present and anastomoses with that of the nearby glomerulus; anastomoses with venulae rectae are also shown at the left (*a*). Rat C-26; × 30.

Fig. 16.—Examples of normal intertubular network for comparison in size with the final net. Part of the long-meshed network, shown at the lower right, has fairly straight segments which give off smaller branches that encircle the tubules. Blood returning after infarction flows mostly in these long straight segments. Rat C-26; × 30.

Fig. 17.—An interlobular artery (→) from the zone of partial necrosis two weeks after infarction showing union with the surrounding capillary network and veins by by-passing the glomeruli. Only the very uppermost glomeruli show partial penetration of the injection fluid. Rat C-73; × 30.

Fig. 18.—The terminal part of an interlobular artery from the zone of partial necrosis at two weeks shows union (→) with the intertubular network by by-passing glomeruli and formation of continuous vessels with reversed bends (*a*). Rat C-73; × 30.

Fig. 19.—Union of branches of an interlobular artery into a hooplike structure is shown (*a*). Rat C-73; × 30.

Fig. 20.—Union of two interlobular arteries in a continuous network with by-passed glomeruli (→). Rat C-73; × 30.

Fig. 21.—Glomeruli from the uppermost layer of the cortical zone of partial necrosis showing only slight filling of capillaries or abrupt cessation of the flow of injection fluid at the glomerular pole. Rat C-82; × 30.

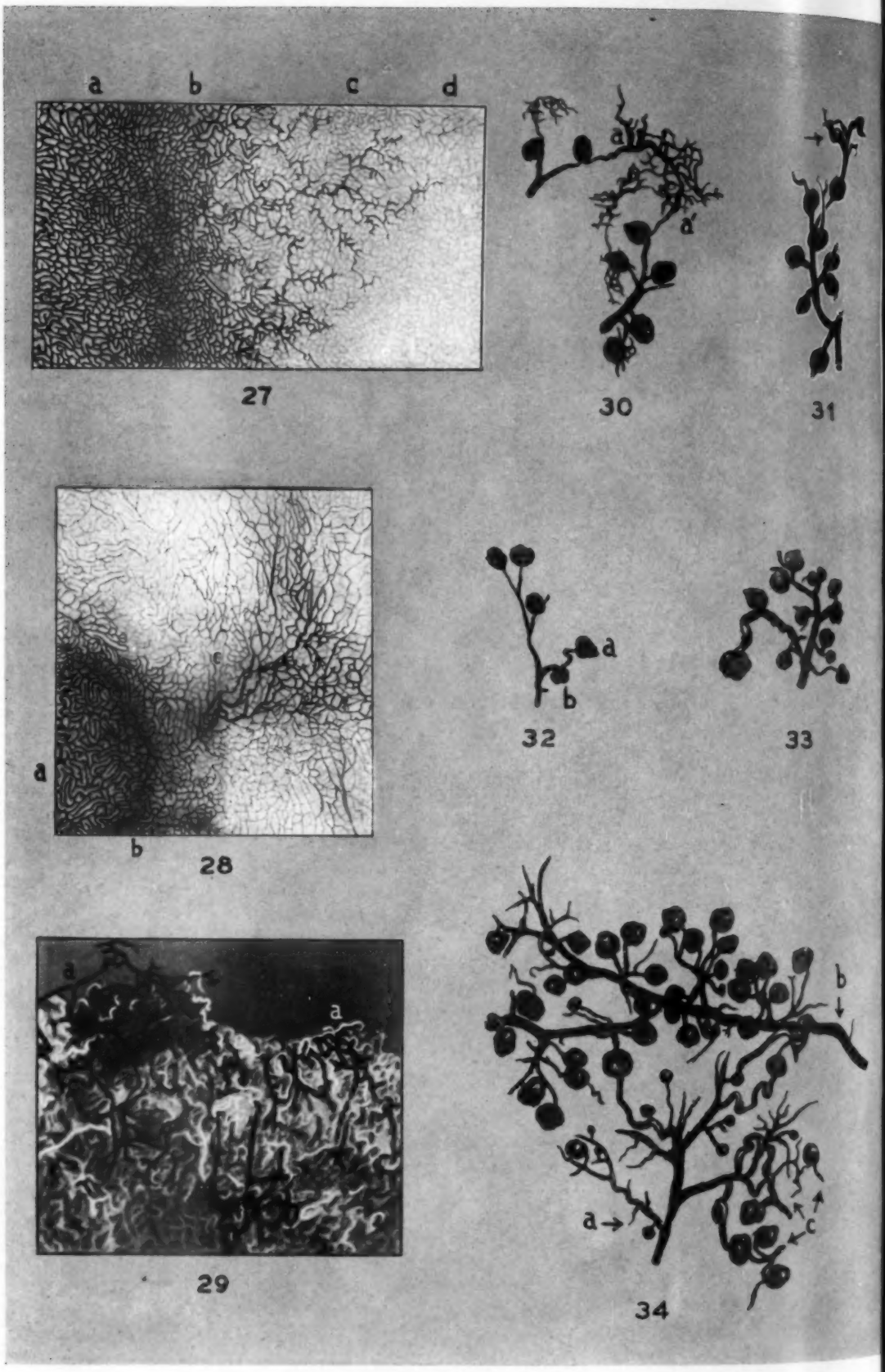
Fig. 22.—New vascular path formed by by-passing an uninjected glomerulus. Rat C-131; × 30.

Fig. 23.—Shrunken but injected glomerulus showing new channels between afferent and efferent arterioles. Rat C-73; × 30.

Fig. 24.—A continuous vessel by-passing a shrunken uninjected glomerulus. Rat C-73; × 30.

Fig. 25.—Network formed by shrunken interlobular arteries united with capillary and venous channels from the center of the infarct after one year. Many of the finest capillary branches have been broken off in dissection. The glomeruli, only a few of which are shown here, are myriads of tiny uninjected bodies caught in this meshwork. Compare this structure for size with that of the normal intertubular capillary network as seen in figures 15 and 16. Rat C-20; × 30.

Fig. 26.—Example of new vascular formation four months after infarction. Note the arteriae rectae verae at the corticomedullary junction, fully injected glomeruli and extremely shrunken uninjected glomerular remains. This dissection also shows the union of the interlobular arteries by a continuous network. Rat C-26; × 30.



Figures 27 to 34

(See legends on opposite page)

The thicket-like appearance of the dilated vessels mentioned in a foregoing paragraph, and the endothelial proliferation which is seen in microscopic sections deeper in the infarct, where regenerating capillaries are both sprouting and regressing, have given rise to the idea that new vessels grow from the periphery of the infarct and "organize" it. This misconception is cleared away by dissection studies, which show the true relation of all these vascular structures.

The changes in the blood vessels of the infarct *at two weeks* (figs. 12 and 13) beyond those observed at one week occur chiefly in the smaller divisions of the arterial system. The formerly unfilled ends of the interlobular branches (fig. 11) are united with the rest of the

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EXPLANATION OF FIGURES 27 TO 34

Fig. 27.—Surface of the cortex three days after infarction. The capillary network at the left (*a*) is normal. Shrinkage is seen in the tubules at the periphery of the infarct (*b*). The flow of injection fluid through the original capillary network on the surface of the infarct is shown in the center (*c*), while the capillaries at the extreme right contain only a minute quantity of ink (*d*). Rat C-118; × 15.

Fig. 28.—Evolution of superficial vessels on the surface of an infarct from the original capillary network observed one week after infarction (*c*). The extreme left hand corner shows the normal pattern (*a*) while at (*b*) is shown the depression due to the shrinkage of tubules in the zones of atrophy and partial necrosis. Rat C-131; × 15.

Fig. 29.—Partially dissected mass of necrotic tissue one month after infarction showing union of interior vessels with those on surface (*a*) and shrinkage of the tubules. Rat C-120; × 15.

Fig. 30.—Distal ends of normal interlobular arteries united by capillary branches of efferent arterioles on the surface of the cortex. Note the thick continuous connection between the two glomeruli (*a-á*). Rat C-26; × 30.

Fig. 31.—Injected interlobular artery from the infarct near the zone of the anterior unligated branch of the renal artery one month after infarction. Branches (→) of the efferent arteriole have become greatly enlarged and twisted as a result of the reversed flow of blood from the anterior system into the interlobular artery. The glomeruli here are almost normal in size. Rat C-122; × 30.

Fig. 32.—Continuous pathway shown at two weeks between a glomerulus of the area supplied by the anterior unligated branch (*a*) and a glomerulus of an area supplied by the ligated posterior branch (*b*). Rat C-73; × 30.

Fig. 33.—Same as figure 32 one year after infarction. Note the size and the twisting of the union (*a*) and of the afferent arteriole. Rat C-111; × 30.

Fig. 34.—Branch from an infarcted area (*a*) united with a branch from the normal area of the anterior branch system (*b*). Note the twists and loops of the connections and the thickened ends of the interlobular artery from the infarcted area. One of these connections appears to have no intervening glomeruli between the branches. The five large glomeruli at the right (*c*) are from another broken-off branch of the anterior system. Rat C-20; × 30.

system by extensions (figs. 12 and 18) that represent the clearing out of original paths and a replacement of the necrotic walls by regenerating endothelial cells and fibroblasts. More filled connections are to be seen uniting main capillary channels with veins and interlobular arteries, the latter sometimes by-passing and sometimes including only part of the glomerulus. Examples of this alteration are shown in figures 17, 18, 19, 20, 22, 23 and 24. Many afferent arterioles contain ink which stops abruptly at the glomerulus (figs. 13, 19 and 21), or the glomerulus may be passed by or included in the channel as a misshapen mass on one side of the vessel.

This shunting arrangement arises as a result of necrosis of some capillary loops without a breakup of the entire glomerulus. A histologic section shows that despite the disappearance of the well defined loop structures and the necrosis of the cells which form them, blood is still being conveyed into the damaged glomerulus (fig. 36).

Other formations not present in the normal vascular architecture develop at this time. The shrinkage of the damaged tissue shortens the distance between glomeruli of the anterior branch system and those of the posterior branch system, and the connections between the two become thicker and more direct (figs. 32, 33 and 34). Such broad connections are seen also between glomeruli within the posterior branch system either connecting tufts on neighboring interlobular arteries or on branches of the same interlobular arteries (figs. 18, 19 and 20). Arteriolae rectae verae and anastomoses of small aglomerular arterioles appear (fig. 26). Intrarenal anastomoses between large branches of the anterior and posterior branch systems as described by Belt and Joelson [12] in the dog were never seen.

Shrinkage of the infarct and the return of the circulation progress together. This shrinkage includes the blood vessels as well as the parenchyma. At two weeks it is noticeable in the glomeruli and vessels at the periphery of the infarct, which from the first had an uninterrupted circulation (fig. 14). It may also be observed in those living tubules and glomeruli near the corticomedullary junction and those close to the surface of the cortex, which are in areas where the blood supply has returned early and is plentiful. The glomeruli and tubules in these regions showed both fatty change and atrophy. The most internal part of the infarct between the two levels just mentioned and farthest away in all directions from the blood supply of the anterior branch of the renal artery is a mass of necrotic tissue which shrinks more slowly.

The vascular arrangements observed at two weeks and more clearly at *one month* (fig. 29) are not essentially altered at later periods, but

a varying decrease in size of structures gives an entirely different appearance to the resulting vascular mass. The main bulk of this is formed by the large divisions of the posterior branch of the renal artery and those arcuate and interlobular arteries with their glomeruli which lie in the region bordering the anterior half of the kidney. These elements, although somewhat shrunken, are seen at the end of *one year* to be of fair size and appear in all examples well injected (figs. 33 and 34). At this time some aglomerular direct connections can be observed between the interlobular arteries of the anterior and posterior systems (fig. 34). The remainder of the vessels of the posterior system diminish to the size of capillaries connected in a complicated network.

The final vascular arrangement is an abundant network of very small vessels lying flattened layer on layer against the fanlike backbone of the larger vessels described earlier. The glomeruli which were hitherto connected with the most interior branches and which have never been included in the circulation after the injury remain enmeshed as tiny bodies in this network (fig. 25), detached from the vessels, along with the small cystic structures, granular particles or calcified remnants that represent the former parenchyma. The connective tissue, which has proliferated chiefly around the blood vessels (fig. 7), forms a very small proportion of the remaining tissue in contrast with the bulk furnished by the vascular elements. This connective tissue containing the parenchymal remnants is indeed so sparse that it seems reasonable to suppose that it represents a condensation rather than a proliferation. Certainly organization of the infarct does not take place as does organization of a thrombus, since the vessels of the infarct and its connective tissue are modified preexisting structures and do not grow in from without. This was pointed out long ago by Langemak [34] and Lange [35] and is the view held by Bell.[36]

THE ROLE OF VARIOUS POSSIBLE AUXILIARY SOURCES OF BLOOD SUPPLY TO THE INFARCTED AREA

Extrarenal Anastomoses, Capsular and Pelvic.—The capillary connections of the obstructed posterior branch with the anterior branch and with other more proximal branches of the renal artery which are found in the pelvis, the ureter, the capsule, the perirenal fat and the adrenal gland bring blood into the posterior renal artery just distal to the obstruction as shown in figure 1, *i, g*. This blood not having passed through glomeruli is at a higher pressure than that in the efferent

34. Langemak, O., in Born, G.; Flügge, K., and others: Bibliotheca medica, Cassel, T. G. Fischer & Co., 1902, pt. C, no. 15.

35. Lange, G.: Frankfurt. Ztschr. f. Path. **6**:185, 1911.

36. Bell, E. T.: Text Book of Pathology, Philadelphia, Lea & Febiger, 1938.

Figure 35

(See legend on opposite page)

arterioles. It might therefore be thought responsible for the preservation of all of the tissue that does not die when the usual circulation is interrupted. That this is not the case is apparent from the fact that the margin of viable tissue near the hilus does not exceed in amount that found in the area supplied by the capillaries in the most distal portion of the circulation (fig. 1 *a*). Spontaneous obstructions that occur from embolism, thrombosis, wounds or surgical procedure are usually more distal than the experimental one produced here, and these inferior branches therefore must play a minor part in a human infarct.

Connections with capsular arteries have been considered responsible for the preservation of the fringe of living tissue which remains for a short time at the surface of the infarct. The greater part of the capsule of the rat's kidney has very few vessels except where the fat joins it at the poles. Here they are numerous, but they are rather in the fat than in the capsule itself. The capsule can be rolled away from the cortex without any evidence of vascular communications. This was also the case with the vessels of the capsule of an infant's kidney, which were carefully examined for communications with vessels in the renal cortex. The capsule of the kidney of a 65 year old man, however, contained vessels which communicated at rare intervals by capillary anastomoses with those on the surface of the cortex. On the other hand, capsular vessels unite with the superficial vessels of the cortex after the production of an infarct, and new capsular vessels are abundantly formed in the event that the capsule has been injured by puncture or tearing, by the formation of a blood clot or by infection. Under these circumstances, the capsule becomes thickened and firmly attached to the tissue beneath it, and the capsular vessels become continuous with those of the cortex. This is a consequence of injury, however, and cannot be significant in the preservation of the underlying tissue nor in the supply of blood to the body of the infarct, because these vessels are being formed and united with those below no faster than the circulation is being established in the cortex itself.

It is evident, therefore, that in the early period of infarction the capsular vessels could play no role in furnishing blood to the under-

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EXPLANATION OF FIGURE 35

Fig. 35.—Dissection of a portion of the renal artery from an infarcted human kidney. The side branches are translucent and carry well preserved, though somewhat atrophied, glomeruli while the center branches are thrombosed and necrotic and bear opaque necrotic glomeruli. Although the obstruction is proximal to all the branches, the side branches (*a*) have been preserved by the influx of blood from contiguous areas supplied by unobstructed arteries. The thrombosis of the interlobular divisions is a result of stasis. Compare with figures 1 and 10. × 15.

lying tissue unless such connections were already numerously established by previous injury. The viable tissue which remains at the surface of the infarct can be supplied, however, by the intertubular capillary network of the cortex corticis (figs. 27 and 30). If only one arcuate branch were cut off from the circulation, there would be enough blood pouring in from all sides by this anastomotic capillary route to fill sufficiently the superficial capillaries so that a layer of living parenchyma could exist at the surface. However, when infarcts as extensive as those in the present experiments are formed, the superficial layer of tissue, which is at some distance from the anterior branch capillary supply, suffers to the same extent as the deeper tissue. This would not be the case if blood were entering it from the capsule at numerous widely distributed points and is further evidence that capsular anastomoses are not present.

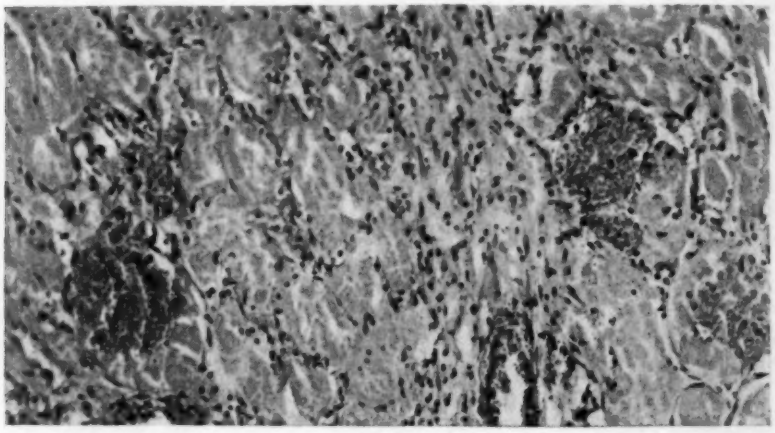

Fig. 36.—Glomeruli from a zone of partial necrosis which contains discrete well preserved erythrocytes but in which the nuclei and the glomerular loops have disappeared. A vessel with patent lumen and discrete erythrocytes is close by. One week after infarction. Rat C-34; × 150.

Influx by Venous Return.—Although we have emphasized the importance of the connections between the anterior and the posterior branch arterial system in furnishing the infarcted area with blood, it might be supposed that venous connections, being numerous and unobstructed by glomeruli, would, as Cohnheim [3] believed, be an important path for the passage of blood back into the infarct. It is true that a certain venous return through the infarcted tissue never completely ceases, but the amount of blood passing through the parenchyma is very limited and is insufficient to prevent injury to the veins themselves. The veins may show fibrin deposition and necrosis of their walls (fig. 6) adjacent to that seen in the arteries. A spreading out of blood into the

intertubular network from this scant venous flow is conceivable where pressure differentials favor it. It is evident, however, that the veins do not independently carry blood to the infarcted tissue, since it is precisely where the arterial perfusion with ink is heaviest that the venous injection is most successful, and where the perfusion fails in the former system, it does in the other. The flow of perfusion fluid and blood in the infarct, therefore, depends on the arterial supply, and the flow of venous blood is locally determined by influx from the neighboring arteries.

Passage of Blood by Vasa Vasorum.—The anastomosing vasa vasorum of an artery are considered by Kurkowsky [33] and Winternitz [29] to play an important part in the conduction of blood into an artery distal to an obstruction in its lumen. They cannot, however, be effective when the artery is severed or even ligated, as in these experiments.

FINDINGS IN NONINJECTED HUMAN INFARCTS

Examination of infarcts from human material reveals the same operating mechanisms as seen in the rat's kidney. No injected material was studied, as it is our experience that postmortem material, even when carefully perfused, is not suitable for detailed study, owing to the blocking of some vessels by postmortem clots and the escape of fluid from capillaries and veins.

As evidence of the reestablishment of circulation in the infarcted area are found well preserved, though atrophied, glomeruli on intact branches of the obstructed artery distal to the point of obstruction. Other antecedent branches are found in the infarct with dilated necrotic segments all occurring at approximately the same level (fig. 35). These arteries, mostly interlobular arteries, contain thrombi which are the obvious result of the damage to the vessels and the accompanying stasis.

Photographs of injected infarcts figured by Gänsslen [37] demonstrate the same point, although his interpretation is a different one. In his figures can be seen thrombi in neighboring interlobular arteries occurring at about the same level; other vessels are made visible by a thin line representing a constricted lumen. Dilatations are also present, and the partial filling of some glomeruli. At the periphery are the well filled branches and glomeruli, surrounded by a thicket of dilated venules that belong to branches of the obstructed vessel contiguous to the area supplied by the unobstructed arteries. These correspond to those in figure 35 mentioned as somewhat atrophied but well preserved.

In human material, in which the point of obstruction is not always found and the time of its occurrence is not known, it is extremely difficult to tell by histologic sections whether the blood seen in the arteries

37. Gänsslen,[10] figures 139 to 142.

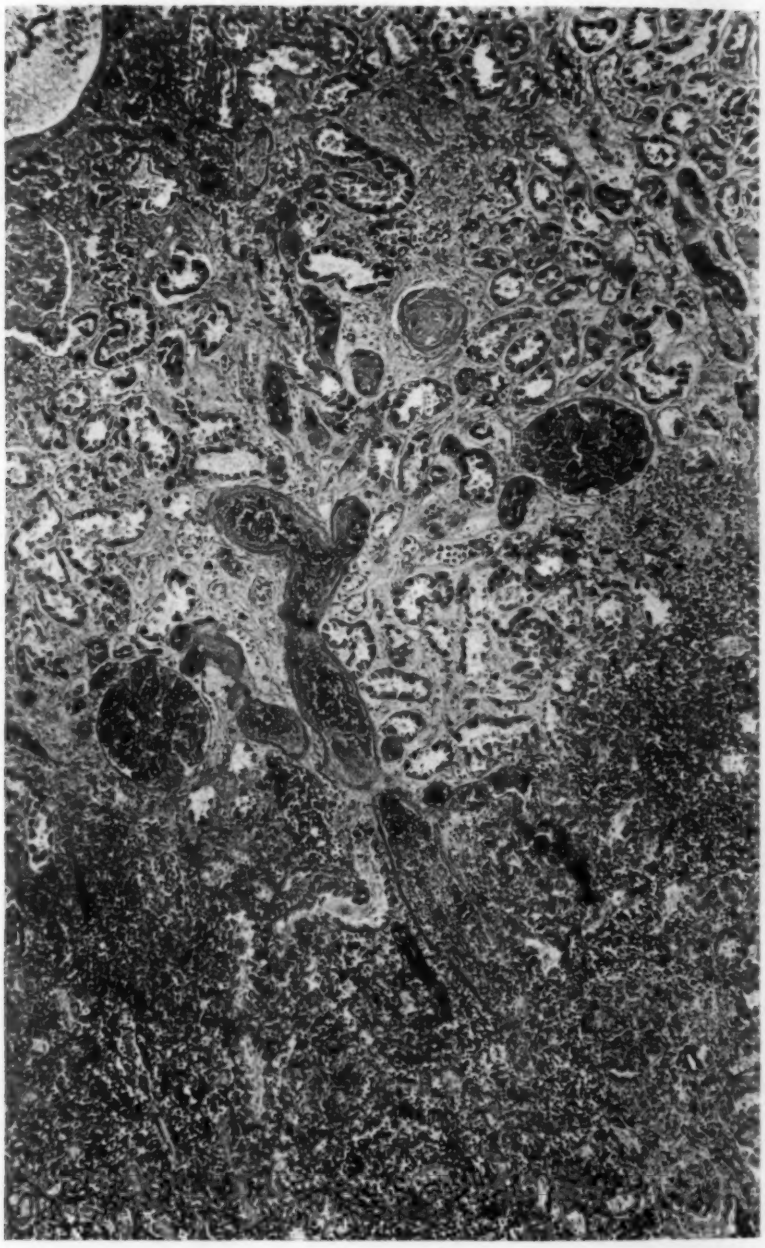

Fig. 37.—An interlobular artery in an infarct in the human kidney showing necrosis of the walls, debris in the lumen, loss of nuclei in the glomeruli but discrete red blood cells in both the artery and the glomeruli. × 80.

is blood which was present at the moment of occlusion and static or blood which was flowing through the infarct from a collateral circulation (fig. 36). Moreover, where other lesions, such as arteriosclerotic scars, are present, the boundary of the tissue suffering injury from the recent obstruction is not obvious. These points can be cleared up only by tracing the blocked vessel to its termination, a procedure difficult and in many instances impossible.

COMMENT

As can be seen from the foregoing presentation, our findings are at variance with the accepted view of the vascular changes which occur after the occlusion of a branch of the renal artery. Instead of complete ischemia occurring, as is commonly supposed, we have demonstrated that the main branches of the obstructed artery and some of the glomeruli function continuously after the obstruction by virtue of blood furnished them through preexisting capillary anastomoses.

The work of Liek [38] and Belt and Joelson [12] on dogs offered the first evidence that a circulation is reestablished in the original vessels of the infarcted area. It is unfortunate, however, that dogs were chosen for experiment because the intrarenal vessels of dogs have more numerous preexisting anastomotic connections than those of other animals (Liek [38]). MacNider [11] in his study on cats pointed out that a colored mass injected into the unligated branch of the renal artery could be seen in the area supplied by the ligated branch three days after obstruction.

Our observations differ, however, from these findings in such particulars as the nature of the anastomoses, the degree of preservation of the glomeruli and the evolution of the final architecture of the circulation of the infarcted area.

MacNider assumed that the arterioles of the medulla but especially arteriae rectae verae, which it is now known are practically nonexistent in young adults, furnished the anastomotic connections between unobstructed and obstructed arteries. According to him, the cortex is supplied at a later date by the growth of new-formed vessels. Belt and Joelson [12] stated in their conclusions "that an almost absolute ischemia confined to the region supplied by the blocked vessel is followed by a late ingrowth of small anastomotic channels into the bloodless area." They did not make clear whether they considered any anastomoses other than those found in the ureter, pelvis and perirenal tissue to have existed before the occlusion. They did state definitely, however, that anastomoses could not be demonstrated between anterior and posterior branches of the renal artery of a normal human kidney when one of these branches was ligated prior to injection.

38. Liek, E.: Virchows Arch. f. path. Anat. **220**:275, 1915.

Another point of difference between our work and that of these investigators concerns glomeruli. MacNider [11] reported new capillaries growing into these bodies and thus effecting a certain revascularization of them that is followed later by fibrosis. Belt and Joelson [12] found that patent glomerular capillaries could be demonstrated in the infarct after two weeks and in increasing number after three and five weeks but not after seven weeks. They did not explain these changes except to offer the opinions of MacNider.

Again, MacNider observed that thirty days after the occlusion of the artery an obliterative endarteritis begins to affect the vessels, the smaller ones especially. Whether he considered this to occur in newly formed vessels or in the old vessels which received blood by anastomoses or in all the vessels is not clear. His conclusion was that the vascular obliteration resulting from this process causes the final scar to have a meager and irregular vascularization.

Our findings in regard to these points may be tabulated as follows.

1. The anastomotic channels are composed of the preexisting capillary connections between efferent arterioles and are an integral part of the intertubular network throughout the renal substance and on the surface of the cortex.

2. The glomeruli nearest an unobstructed blood supply receive blood from it from the first and persist as part of the circulation for at least a year after infarction. These glomeruli do not reenter the circulation as a result of revascularization nor are they cut off from it by fibrosis but are in continuity with it from the outset. The remainder of the glomeruli of the infarct either become partially necrotic and later show fibrosis, or, as happens to the majority, become completely necrotic and dwindle to minute acellular spheres detached from the vessels (fig. 25).

3. There is an early fibroblastic proliferation in and around the arteries (figs. 5 and 7), especially evident about those larger ones in which earlier there were edema and infiltration of erythrocytes into the vascular walls. These exudative changes are followed by hemosiderin and fat deposition and proliferative changes.

4. The scar of the infarct instead of being poorly vascularized consists almost entirely of very densely crowded vessels. These fine vessels are not ordinarily seen in histologic section, probably because they readily collapse as do the channels of a telangiectasis whose appearance is often that of a solid mass of tissue.

During the course of the adaptation of the circulatory paths to alterations in direction and amount of flow of blood and to the loss of tissue which occurs as a result of arterial occlusion, a number of interesting changes occur which have been noted in the body of this paper but which merit fuller discussion.

The investigations of Clark and co-workers [39] showed the extent to which a remodeling of a vascular network can take place. These investigators found in living preparations that capillaries especially, but also veins and even arteries, are subject to changes in size, shape and distribution by modifications in blood flow and changing tensions inside and outside the vessels. Their demonstration of the lability exhibited by the capillaries in growing and healing tissue supports our observation of the evolution of the vessels on the surface of the infarct into a pattern different in appearance but nevertheless remodeled from the original intertubular capillary network. It explains the simplification of this network into a system of vessels in the depths of the necrotic renal substance less branched than the continuously dividing and uniting meshwork of the normal capillary bed. Dilatation of these small channels at the outset aids in the flow of blood from the open circulation to that closed by obstruction. There is a selective routing of the blood flow by way of the easiest course with a consequent change in the architecture of the network by the progressive enlargement of some portions of it and the loss of unused parts (Clark).[40]

Why blood does not spread immediately through the entire capillary network of the infarcted area has been explained variously by different observers. Karsner and Austin [6] suggested that thrombi form in the capillaries; Ribbert,[5] that the capillaries become plugged with polymorphonuclear leukocytes, and Beattie and Dickson,[41] that the endothelium swells and causes obstruction to blood flow. We feel that the fundamental explanation lies in the small amount of blood available, the insufficient pressure and the resistance offered by the narrow channels. As the blood which was formerly distributed by the occluded branch of an artery flows into the unobstructed branches it is not distributed selectively to the terminals nearest the injured area but proportionately to all ultimate units, so that the only extra blood that is furnished to the infarcted area is that which passes through the capillaries which are immediately contiguous to it. This represents a very small part of that which was formerly carried to the infarcted area by the obstructed artery. The factors suggested by the workers just mentioned would be rather consequences than causes of the initial failure of the blood to move freely to any great distance into the infarct, but later these factors may arise and operate.

The ability of the available blood to pass into the infarct only slowly and unevenly gives rise to variation in the alterations observed as one

39. (*a*) Clark, E. R.; Hitschler, W. J.; Kirby-Smith, H. T.; Rex, R. O., and Smith, J. H.: Anat. Rec. **50**:129, 1931. (*b*) Clark, E. R., and Clark, E. L.: Am. J. Anat. **64**:251, 1939.

40. Clark and others,[39a] figures 3 and 4. Clark and Clark,[39b] figure 8.

41. Beattie, J. M., and Dickson, W. E. C.: Textbook of Pathology, ed. 3, St. Louis, C. V. Mosby Company, 1926.

passes from the outermost to the innermost portions of the infarct. There is variation in the density of the necrotic tissue and the rate of its absorption. We have already mentioned differences observed in glomeruli in different locations.

Certain new vascular formations which have previously been described by one of us [18] in arteriosclerotic scars, and whose progressive development has been studied by MacCallum [42] in a series of animal kidneys, are readily observed in the zone of partial necrosis. We refer to the development of aglomerular branches of the interlobular arteries (Ludwig's vessel). They also arise from the arcuate arteries and pass into the medulla as arteriae rectae verae. All these direct branches are a result of changes in the glomeruli, such as necrosis of capillaries or fibrosis, which prevent circulation of blood through the tuft. A by-pass which may partially enter the glomerulus is thus effected, and a communication is established between the afferent and efferent arterioles. These direct branches are found in great number near the edge of the infarct, and their appearance gives further weight to the accumulating evidence that such variants of the usual renal architecture are the result of the evolution of the vascular pattern that comes as effects of normal aging or fortuitous disease.[20]

Recognition of the evolutionary adaptation of the vascular bed that follows occlusion of a branch of the renal artery suggests certain practical speculations.

After occlusion of a branch of the renal artery, the blood must be carried by the other branches, and this added pressure is present from the first moment of occlusion. Because of it, blood is carried into the ischemic tissue by already existing pathways, which enlarge with use. An appreciation of these facts suggests a possible treatment of local disturbances of circulation that threaten ischemic damage. If the current notion that reestablishment of circulation must await the ingrowth of new vessels is discarded, favorable influences might be brought to bear so that capillary paths may be dilated, blood flow increased, pressure raised and blood prevented from clotting. Heparin, plethora, diathermy, blood pressure elevation, temperature elevation and oxygen saturation are factors that might act in this direction.[43]

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42. MacCallum, D. B.: Am. J. Anat. **65**:69, 1939.

43. Such a concept may well have applications beyond the field of renal abnormalities. It would seem to be particularly applicable to coronary occlusion in which the onset is more evident and the tissue less susceptible to anoxemic death than in obstruction of a renal vessel. Schlesinger (Am. Heart J. **15**:528, 1938) showed that no large anastomoses between coronary arteries in the normal human heart can be demonstrated by his methods. There must, therefore, be either capillary anastomoses or none. In the light of our work we are inclined to believe that capillary anastomoses capable of enlargement exist here as elsewhere.

It seems certain that the structural modifications in the vascular bed of the infarct must have functional significance. As will be described in a later study, there is little parenchymatous structural restitution in the infarcted area, so that the eliminatory functional return is mimimal. The continuous flow of blood through the infarcted area offers, however, an opportunity for the introduction into the circulation of products of ischemic renal tissue which have, as the Goldblatt experiments demonstrated, a pronounced physiologic effect on the circulation. We have shown that the circulation in the infarct brings the blood into contact with both partially damaged and completely necrotic tissue. What effect variation in the relative preponderance of these tissue changes may produce as the infarct heals is now being investigated.

CONCLUSION

The vascular changes that occur as a result of interference with the blood supply in the kidney are conditioned by the original and peculiar design of its circulatory system. Though ultimately this architecture becomes greatly altered, the evolution of its final form can be traced step by step from the original pattern.

NORMAL AND ABNORMAL MITOTIC ACTIVITY

I. COMPARISON OF PERIODIC MITOTIC ACTIVITY IN EPIDERMIS, RENAL CORTEX AND SUBMAXILLARY SALIVARY GLAND OF THE ALBINO RAT

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The factors which control mitosis determine how often and when it occurs. Therefore, rate and periodicity are ultimate expressions of the regulation of mitotic activity, and investigation of them may be of great assistance in the analysis of neoplastic and other forms of abnormal growth.

Although many aspects of mitosis have been examined, few studies have been made of its rate and periodicity in the animal organism. A careful search of the literature showed eleven reports on this subject. Fortuyn–van Leyden,[1] Ortiz Picón,[2] Carleton,[3] Cooper and Schiff,[4] Blumenfeld,[5] Cooper,[6] Broders and Dublin[7] and Cooper and Franklin[8] have found diurnal periodicity of mitotic activity in certain organs of the cat, the mouse, the rat and man. Elliott[9] did not find periodicity, but he obtained specimens only twice in the twenty-four hour periods. The unanimity of findings indicates that mitotic activity is periodic in animals.

Certain of the factors which govern mitosis must be responsible for its daily rise and fall in rate. In attempting to learn the nature of these factors by studying differences related to the diurnal variation, it is essential to determine whether the twenty-four hour curve of mitotic activity is the same or different for each of the organs of the body.

From the departments of pathology of Cleveland City Hospital and Western Reserve University.

1. Fortuyn-van Leyden, C. E. D.: Verhandl. d. k. Akad. v. Wetensch. Amsterdam **19**:38, 1917; **29**:979, 1926.
2. Ortiz Picón, J. M.: Ztschr. f. Zellforsch. u. mikr. Anat. **23**:779, 1933.
3. Carleton, A.: J. Anat. **68**:251, 1934.
4. Cooper, Z. K., and Schiff, A.: Proc. Soc. Exper. Biol. & Med. **39**:323, 1938.
5. Blumenfeld, C. M.: Anat. Rec. **72**:435, 1938; Science **90**:446, 1939.
6. Cooper, Z. K.: J. Invest. Dermat. **2**:289, 1939.
7. Broders, A. C., and Dublin, W. B.: Proc. Staff Meet., Mayo Clin. **14**:423, 1939.
8. Cooper, Z. K., and Franklin, H. C.: Anat. Rec. **78**:1, 1940.
9. Elliott, H. C.: Am. J. Anat. **58**:127, 1936.

This is important because, first, it will shed some light on the nature of the factors, and, second, it will indicate the optimum time at which to obtain material for studying differences. The first premise may be explained further. Thus, if the factors which regulate mitosis act on or reside within each cell as a unit, regardless of location or type of cell, then, according to the law of chance, periodicity of mitotic activity will not be found consistently in an organ. But periodicity of mitotic activity has been demonstrated repeatedly in several organs. Therefore, it may be assumed that regulation of mitosis is not exhibited by the cell as the unit. If the factors which regulate mitosis are of a general nature, acting on the entire organism at about the same time, it may be expected that all organs will exhibit a more or less similar periodicity of mitotic activity, and the search may be directed toward such factors as light, temperature, time of feeding, muscular activity and a hormone. If the factors which regulate mitosis act on or reside within the organ as a unit, it may be expected that each organ will exhibit a different periodicity of mitotic activity, and the search for the factors which determine how often and when mitosis occurs may be directed toward the organ. For these reasons the present study, a comparison of periodic mitotic activity in organs from three different systems of the albino rat (the renal cortex, the epidermis and the submaxillary salivary gland), has been made. The observations on kidney and epidermis have been reported previously.[5] The findings in the submaxillary gland will be presented first; next, the comparison of the periodicity of mitotic activity in the three organs.

MATERIALS AND METHODS

Details having been given in preceding papers,[5] only the main points are stated. The organs were obtained from 96 male albino rats of the Wistar strain. When 28 days old, they were killed at successive intervals of two hours, resulting in twelve groups of 8 animals each. The organs were fixed in Bouin's fluid within five minutes, embedded in paraffin, sectioned at 8 microns and stained with Harris' hematoxylin and eosin Y. Mitotic activity was determined by counting the mitoses in 1,000 fields at a magnification of 1,000 diameters. A field was an arbitrary area demarcated by four hairs in the eye piece. After mitotic activity had been determined in the first 30 submaxillary glands, a statistical comparison of results based on the first and second 500 fields showed no significant difference. Thereafter, only 500 fields per gland were examined. In comparing mitotic activity in the submaxillary gland with that in the kidney and that in the epidermis, values derived from the study of 500 fields were doubled since the previous work on kidney and epidermis was on the basis of 1,000 fields per specimen.

The method of counting mitoses in fields has important advantages over that of counting nuclei, employed by most of the investigators cited, and that of whole mounts, used by Cooper and her co-workers. It is simple, rapid and far less tiresome than counting nuclei. Those using the method of counting nuclei usually

observed 5,000 to 15,000 or less. In the submaxillary salivary gland there are approximately 60 nuclei per field used in these studies. The examination of 500 fields means the study of 30,000 nuclei. Thus, with less time, a larger and therefore more representative portion of an organ can be studied. With the method of fields the volume of tissue examined is known, permitting estimates of mitoses per unit volume or per organ and comparison of mitotic activity in different organs and in tumors. The method of whole mounts cannot be applied well to markedly hairy skin, and neither the number of mitoses per nuclei nor the number per volume can be estimated readily.

Fisher's small sample methods [10] for groups of 8 values and standard methods [11] for groups of 24 or more values were employed in evaluating differences between means and coefficients of correlation.

RESULTS

Submaxillary Salivary Gland.[12]—Individual values are listed in table 1 and presented graphically in the accompanying figure. The curve is

TABLE 1.—*Mitoses per Five Hundred Fields in the Submaxillary Salivary Gland of the Albino Rat*

8-10 P.M.	10-12 P.M.	12-2 A.M.	2-4 A.M.	4-6 A.M.	6-8 A.M.	8-10 A.M.	10-12 A.M.	12-2 P.M.	2-4 P.M.	4-6 P.M.	6-8 P.M.
92	44	21	11	50	66	23	26	20	61	27	36
10	13	56	8	17	6	100	71	32	39	47	30
26	22	29	21	20	25	22	23	19	48	36	26
9	20	24	9	12	32	44	39	12	47	24	13
23	56	32	5	31	22	21	43	17	13	55	53
19	67	49	19	21	56	8	10	60	33	15	34
46	81	18	15	42	50	30	8	7	11	33	19
18	15	32	4	14	17	22	2	20	46	10	21
30.3	39.7	30.1	11.5	25.8	34.2	33.7	27.7	23.3	37.2	30.8	29.0

composed of mean values for each two hour interval, placed midway in the segment representing that interval. The individual values for each two hour interval show wide variation. Mitotic activity was minimal between 2 a. m. and 4 a. m. Comparison of the minimal value with those of other two hour periods (table 2) revealed significant differences for all except the 8 to 10 p. m., 10 a. m. to 12 m. and 12 to 2 p. m. intervals; and for these the differences border on significance. Comparisons of the mean values of all other intervals showed no significant differences.

For comparison of activity during the day and night, the twenty-four hour period was divided thus: day, 8 a. m. to 8 p. m.; night, 8 p. m. to

10. Fisher, R. A.: Statistical Methods for Research Workers, ed. 7, Edinburgh, Oliver & Boyd, 1938.

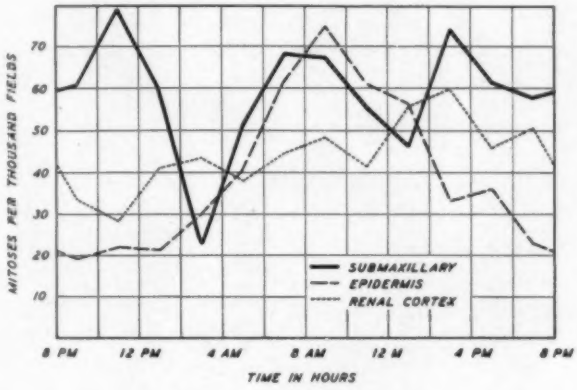
11. Pearl, R.: Introduction to Medical Biometry and Statistics, ed. 3, Philadelphia, W. B. Saunders Company, 1940.

12. This portion of the work was done with the assistance of Helena Rice Glatt, supported by a grant from the Utah chapter of the American Association of University Women.

8 a. m.; first half of day, 8 a. m. to 2 p. m.; second half of day, 2 p. m. to 8 p. m.; first half of night, 8 p. m. to 2 a. m.; second half of night, 2 a. m. to 8 a. m. From the data given in table 2 it is seen that none of the differences is significant. Mitotic activity during the second half of the night approximates a significantly lower level, obviously due to the slight activity between 2 and 4 a. m.

From these data it is seen that mitosis in the submaxillary salivary gland exhibited a significant periodic diminution in rate at 2 to 4 a. m., and a fairly constant level, with insignificant variations, during the remainder of the twenty-four hour period.

Comparison of Mitotic Activity in the Submaxillary Salivary Gland, Epidermis and Renal Cortex.—The curves representing the rate and periodicity of mitotic activity in the three organs studied (figure) show few similarities. Maximal activity occurs at 8 to 10 a. m. in the



Curves of mitotic activity in the submaxillary salivary gland, epidermis and renal cortex for a twenty-four hour period.

epidermis and 2 to 4 p. m. in the renal cortex. There is no special maximal period in the submaxillary gland. Minimal activity occurs at night in all: epidermis, 6 to 12 p. m.; renal cortex, 10 to 12 p. m.; submaxillary gland, 2 to 4 a. m. Thus, the curves differ in character. In epidermis, maximal and minimal two hour periods are separated by equal intervals of ten hours. In the renal cortex there is a gradual rise from minimal activity for fourteen hours and an abrupt decline in six hours. In the submaxillary gland there is a fairly constant level of mitotic activity throughout the day and night, except for an abrupt decline to and rise from the 2 to 4 a. m. period of minimal activity. Thus it is seen that each of the organs studied exhibits a different form of periodicity of mitotic activity.

Is mitotic activity in one organ related to or influenced by that in another? Does it exhibit any relation to body weight? The results of a study of correlation are given in table 3. Perfect correlation is repre-

TABLE 2.—*Comparison of Mean Values of Mitotic Activity in the Submaxillary Salivary Gland for Certain Intervals of the Day and Night*

Time	Mean		Time	Mean	Diff. ± S.E.*	P †
Two Hour Intervals—8 Animals per Group						
2 a.m. - 4 a.m.	11.5	vs.	4 a.m. - 6 a.m.	25.8	14.3 ± 2.66	0.02 - 0.01
2 a.m. - 4 a.m.	11.5	vs.	6 a.m. - 8 a.m.	34.2	22.7 ± 2.94	0.02 - 0.01
2 a.m. - 4 a.m.	11.5	vs.	8 a.m. -10 a.m.	33.7	22.2 ± 2.14	0.05 - 0.02
2 a.m. - 4 a.m.	11.5	vs.	10 a.m. -12 m.	27.7	16.2 ± 1.94	0.10 - 0.05
2 a.m. - 4 a.m.	11.5	vs.	12 m. - 2 p.m.	23.3	11.8 ± 1.87	0.10 - 0.05
2 a.m. - 4 a.m.	11.5	vs.	2 p.m. - 4 p.m.	37.2	25.7 ± 3.90	Less than 0.01
2 a.m. - 4 a.m.	11.5	vs.	4 p.m. - 6 p.m.	30.8	19.3 ± 3.31	Less than 0.01
2 a.m. - 4 a.m.	11.5	vs.	6 p.m. - 8 p.m.	29.0	17.5 ± 3.57	Less than 0.01
2 a.m. - 4 a.m.	11.5	vs.	8 p.m. -10 p.m.	30.3	18.8 ± 1.88	0.10 - 0.05
2 a.m. - 4 a.m.	11.5	vs.	10 p.m. -12 p.m.	39.7	28.2 ± 2.97	0.02 - 0.01
2 a.m. - 4 a.m.	11.5	vs.	12 p.m. - 2 a.m.	30.1	18.6 ± 4.54	Less than 0.01
Six Hour Intervals—24 Animals per Group						
8 p.m. - 2 a.m.	33.4	vs.	2 a.m. - 8 a.m.	23.8	9.6 ± 5.59	1.71
8 p.m. - 2 a.m.	33.4	vs.	8 a.m. - 2 p.m.	28.2	5.2 ± 6.30	0.82
8 p.m. - 2 a.m.	33.4	vs.	2 p.m. - 8 p.m.	32.3	1.1 ± 5.33	0.20
2 a.m. - 8 a.m.	23.8	vs.	8 a.m. - 2 p.m.	28.2	4.4 ± 5.68	0.77
2 a.m. - 8 a.m.	23.8	vs.	2 p.m. - 8 p.m.	32.3	8.5 ± 4.58	1.85
8 a.m. - 2 p.m.	28.2	vs.	2 p.m. - 8 p.m.	32.3	4.1 ± 5.43	0.75
Twelve Hour Intervals—48 Animals per Group						
8 a.m. - 8 p.m.	30.2	vs.	8 p.m. - 8 a.m.	28.6	1.6 ± 3.98	0.40

* S. E. signifies standard error.

† P for groups of 8, calculated by Fisher's [10] methods, expresses the probability of occurrence of like differences by chance, e. g., a value of 0.01 means that by chance such a difference would occur once in one hundred times. Conventionally, P greater than 0.05 is interpreted as indicating no significance. P for groups of 24 and 48 is a significance ratio calculated by standard methods (Pearl [11]). Conventionally, a P of 2.0 or more is interpreted as indicating statistical significance because there is less than one chance in twenty that such a difference could occur by chance.

TABLE 3.—*Correlation Between Mitotic Activity in the Submaxillary Gland, the Renal Cortex and the Epidermis, and Body Weight*

Comparison	Coefficient of Correlation ± S.E.
Submaxillary gland and epidermis	0.163 ± 0.103
Submaxillary gland and renal cortex	0.073 ± 0.102
Submaxillary gland and body weight	0.141 ± 0.102
Epidermis and renal cortex	0.257 ± 0.104
Epidermis and body weight	0.119 ± 0.104
Renal cortex and body weight	−0.210 ± 0.103

sented by a coefficient of 1.0. The highest coefficient obtained, 0.257 ± 0.104 for skin and kidney, although differing significantly from zero, indicates little correlation. It may be concluded that there was no correlation in mitotic activity between the various organs, nor any correlation between mitotic activity and body weight.

COMMENT

The observations presented show that in the submaxillary salivary gland, the epidermis and the renal cortex of the albino rat, mitotic activity exhibits diurnal periodicity. This is in agreement with the findings of all of the investigators cited except Elliott, whose observations were too limited to constitute a contrary finding. The periodicity is different for each of the three organs studied. Mitotic activity is at an ebb during the night. The rat is a nocturnal animal, which immediately suggests cessation of vegetative activity during the period of greatest functional activity as the explanation. However, no general influence acting on the entire body can explain maximal activity in the epidermis in the morning, in the renal cortex during the afternoon and in the submaxillary throughout most of the day and night. Whatever the factors that regulate mitosis may be, those immediately concerned with when and how often it occurs act on or reside within the organ as a unit.

The application of these results to studies which concern themselves with factors regulating mitosis or with periodicity of mitotic activity is important. Blumenthal [13] concluded from a study of mitotic activity in thyroid and adrenal glands of guinea pigs that metabolic processes initiated by intake of food are probably responsible for a rise in number of mitoses. Such a conclusion is not warranted until the curve of activity under ordinary circumstances is established for these organs, and it is inconsistent with the present observation in the rat of different curves of activity in the three organs examined. Dublin, Gregg and Broders,[14] reporting on the absence of difference in mitotic activity in portions of 5 tumors (adenocarcinoma) of the large intestine at 10 to 12 m. and 10 to 12 p. m., stated "One may expect them also to grow with no consideration for the law of rhythm which we have reason to believe governs normal tissues." Since the study here reported indicates a different rhythm for each of three organs, until the rhythm of mitotic activity in the human colon is established it cannot be determined at what time to remove portions of neoplasms to test their disobedience of the "law of rhythm." The studies of Thuringer [15] on the effect of mechanical and electrical stimulation on mitotic activity in the epidermis of the cat's paw showed a peak two hours after stimulation was begun, then a decline. No statement is made as to the time of initiation of the experiment or the biopsy. Since mitotic activity has been shown to be

13. Blumenthal, H. T.: Endocrinology **27**:481, 1940.

14. Dublin, W. B.; Gregg, R. O., and Broders, A. C.: Proc. Staff Meet., Mayo Clin. **15**:623, 1940; Arch. Path. **30**:893, 1940.

15. Thuringer, J. M.: J. Invest. Dermat. **2**:313, 1939.

periodic in the epidermis of man, the rat and the mouse, it is probably so in the cat; and the observations of Thuringer cannot be evaluated unless compared with the curve of normal mitotic activity.

In general, it may be stated that any study of the rate and the rhythm of mitotic activity must be controlled by a study of the average rate and the average rhythm of the same structure; that deductions as to rate and rhythm, in view of the marked individual variation, must be based on adequate sampling, subjected to statistical analysis, and applied not to the individual but to the group.

SUMMARY

Periodicity of mitotic activity was found in the submaxillary salivary gland, the epidermis and the renal cortex of the albino rat.

The twenty-four hour curve of mitotic activity differed for each organ. There was slight or no correlation of mitotic activity in these organs, or between mitotic activity in them and body weight.

The observations suggest that the factors which determine when and how often mitosis occurs act on or reside within the organ as a unit.

FIBROUS DYSPLASIA OF BONE

A CONDITION AFFECTING ONE, SEVERAL OR MANY BONES, THE GRAVER CASES OF WHICH MAY PRESENT ABNORMAL PIGMENTATION OF SKIN, PREMATURE SEXUAL DEVELOPMENT, HYPERTHYROIDISM OR STILL OTHER EXTRA-SKELETAL ABNORMALITIES

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In 1938, in an article entitled "Polyostotic Fibrous Dysplasia",[1] one of us (L. L.) attempted to bring clarity into the confused situation obtaining in regard to the condition in question, instances of which had been reported previously under many different names. That article gave clinical, roentgenographic and pathologic details of 4 cases studied in this hospital (supplemented by information on 4 in other hospitals) and was directed toward showing that the condition constitutes a definite disease entity. The description of the pathologic alterations of bone which it supplied seemed to us to permit a sounder interpretation of the roentgenographic findings and in general to establish firmly the character of the skeletal changes constituting the nucleus of the disease. Since then, observations in 15 additional cases have accumulated (9 in this hospital and 6 in other hospitals), so that our total experience with the disorder now covers 23 cases. In all of these we had at our disposal not only the history and the clinical findings but the roentgenograms (often covering the entire skeleton). Furthermore, we were able to make a microscopic examination of tissue from one or more bone lesions in all these 23 cases, so that in every instance the diagnosis was established also on an anatomic basis. The present paper serves the purpose of recording our broadened experience in relation to the growing literature on the subject which has developed during the past few years on the basis of the articles of McCune and Bruch,[2] Albright and associates [3] and Lichtenstein.

From the Laboratory Division of the Hospital for Joint Diseases.

1. Lichtenstein, L.: Arch. Surg. **36**:874, 1938.

2. McCune, D. J., and Bruch, H.: Am. J. Dis. Child. **54**:806, 1937. McCune, D. J.: ibid. **52**:734, 1936.

3. (*a*) Albright, F.; Butler, A. M.; Hampton, A. O., and Smith, P.: New England J. Med. **216**:727, 1937. (*b*) Albright, F.; Scoville, W. B., and Sulkowitch, H. W.: Endocrinology **22**:411, 1938.

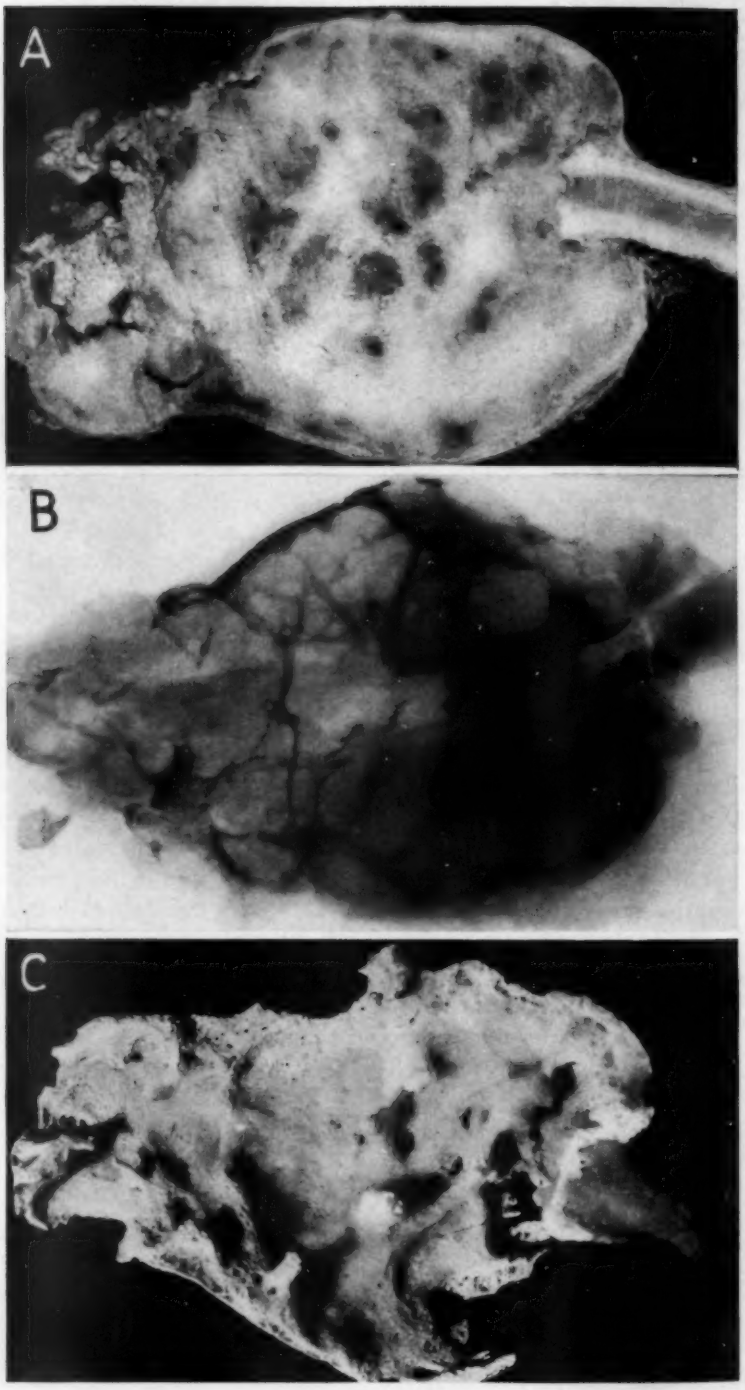

Figure 1

(See legend on opposite page)

As stated in the subtitle, one, several or many bones may be involved in any particular case of fibrous dysplasia. When more than one bone is affected, the involvement tends to be unilateral or predominantly unilateral. In an affected bone, the area implicated may be rather limited or very extensive. The contour of the bone as a whole may still be found normal but is more likely to be found distended in all or at least part of the affected area. Even when not expanded, the cortex is likely to be found thinned by erosion from the medullary side. In the area involved, the interior of the bone is found to be filled mainly by a rather rubbery and compressible tissue, which may be more or less uniformly whitish or may show reddish speckling where there is vascular dilatation and congestion and perhaps capillary hemorrhage. Fundamentally, this tissue is fibrous connective tissue. It may be gritty throughout from the presence everywhere in it of newly formed trabeculae of immature bone. On the other hand, it may show some smaller or larger nongritty, highly collagenous areas in which few, if any, bony trabeculae are to be found. In some lesions, islands of hyaline cartilage may also be present within the fibrous connective tissue. Furthermore, in an occasional lesion, focal degeneration of this tissue may have led to the formation of small secondary cysts.

The cases we have seen do not include many in which the skeletal involvement was dramatically severe, although such cases are rather heavily represented among the published instances of the disease. On the other hand, we have seen relatively many of the cases in which the disease was of a milder type (notably those showing involvement of only one bone or at most a few bones). These cases, and especially those

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EXPLANATION OF FIGURE 1

A, photograph of a resected portion of an affected rib, showing cut surface of the bisected specimen. Note the costal cartilage to the left, around and beyond which the rib has undergone tremendous expansion. Particularly above, and also to some extent below, a thinned cortical shell is visible. The grayish mottled mass represents the gritty fibrous tissue constituting the body of the lesion. The specimen was part of the left seventh rib of a woman of 35 in whom several other ribs were also affected (see fig. 10*A*).

B, roentgenogram of the specimen shown in *A*. Note that the cortical shell is attenuated at best and missing entirely in some places. Observe also that the body of the lesion presents a loculated or trabeculated appearance, explained by *C*.

C, photograph of the costal cartilage and cortical shell remaining after maceration of the specimen shown in *A*. The defects in the cortical shell represent areas where the cortex was actually missing or where it was so thin that it came away when the specimen was macerated. Note the presence of short bony spurs and low ridges on the endosteal contour of the cortex. It is they that impart the trabeculated or pseudoloculated appearance to the roentgen picture of the lesion.

showing involvement of one bone only, are, we believe, underrepresented in the literature, being often interpreted as instances of "localized osteitis fibrosa" and therefore remaining unreported as not being of special interest. Yet we feel quite sure, after studying the bone lesions in all forms of the disorder, that the milder condition which we have observed in older children and in adults and the very severe condition appearing mainly in infants and younger children, as described, for instance, by Goldhamer,[4] Borak and Doll [5] McCune and Bruch, and Albright and associates, represent merely different expressions of the same basic disease. The skeletal lesions, however variable their extent and severity, appear to be analogous in all the cases, thus constituting the common factor of the disease.

The extraskeletal abnormalities manifest themselves principally in the cases in which the skeletal lesions are relatively severe and extensive, and only occasionally and sporadically in the less severe or milder forms of the condition. They include: pigmentation of the skin, endocrine dysfunctions (premature sexual development in females, hyperthyroidism) and premature skeletal growth and maturation. Moreover, a number of recent observations indicate that cardiovascular and renal developmental anomalies may also be associated with the condition. In all probability, the complete story of these associated changes has not yet been told. Indeed, we rather expect that, as more clinical and autopsy material becomes available, this already large list of nonskeletal abnormalities will grow. In fact it seems very probable that fibrous dysplasia of bone, like Recklinghausen's neurofibromatosis, represents potentially another museum of developmental abnormalities.

The originally expressed conception of the disorder (a conception to which we still adhere) is that the condition as a whole represents a congenital anomaly of development. (We cannot accept the idea of Bremer [5a] that cases such as are being described here may have a basis in the effects of long-continued secretion of estrogen, probably acting through the parathyroid glands.) As to the skeletal lesions in particular, these apparently result from perverted activity of the specific bone-forming mesenchyme, producing a focus or foci of involvement in one bone, a few bones or many bones. In a sense, the individual bone lesion may be conceived as a hamartoma—i. e., a tumor-like malformation resulting from flaws of development and characterized by defects of tissue combination. We have tried to suggest this interpretation of the derivation of the bone lesions by the name "fibrous dysplasia of bone."

Indeed, this name is also appropriate for the disease as a whole, since the skeletal lesions constitute the central feature of the clinical syndrome

4. Goldhamer, K.: Fortschr. a. d. Geb. d. Röntgenstrahlen **49**:456, 1934.
5. Borak, J., and Doll, B.: Wien. klin. Wchnschr. **47**:540, 1934.
5a. Bremer, J. L.: Arch. Path. **32**:200, 1941.

and it is mainly in some of the graver cases that nonskeletal abnormalities are present in addition. It would seem that features of a disease which are not consistently found should hardly be included in its name if this name is to apply to all cases. On the other hand, one could argue that the cases showing involvement of only one bone or a few bones and no extraskeletal changes present no indications of a basis in a deep-rooted defect of development. However, from the standpoint of pathology there is an essential uniformity about the bone changes in all instances, irrespective of the extent of the skeletal involvement, which seems to relate all the cases. Furthermore, one can cite neurofibromatosis, for instance, as a precedent both for our choice of nomenclature and for our integration of the mild with the severe forms of fibrous dysplasia into a common concept. Specifically, on one hand, a case presenting merely two or more neurofibromas along the course of peripheral nerves is already designated as one of neurofibromatosis. On the other hand, observers still classify under the head of neurofibromatosis, without qualifications, a case showing hundreds of neurofibromas along the course of nerves, brownish pigmentation of the skin, congenital skeletal abnormalities (spina bifida, scoliosis, congenital fusion of vertebrae, complete or partial absence of a limb bone), circumscribed giantism of visceral parts (particularly of the gastrointestinal tract), mental deterioration and other abnormalities. Indeed, it is recognized that such cases of neurofibromatosis represent merely the most florid expressions of a disorder generally accepted as a deeply rooted hereditary and even familial disease.

We have been able to find thirty-three other titles under which relevant cases have been described. Most of these titles represent variations on the theme of "osteitis fibrosa" or "osteodystrophia fibrosa," sometimes qualified by such terms as "focal," "unilateral," or "disseminated" and often also supplemented by references to some of the extraskeletal abnormalities.[6] Most of the rest of the titles ring the changes on "fibrocystic disease of bone" or on "a form of Recklinghausen's disease of bone," which names are often likewise qualified by such terms as "regional" and "unilateral" and sometimes likewise supplemented by references to associated extraskeletal changes.[7] However, "fibrous dysplasia

6. (*a*) Hunter, D., and Turnbull, H. M.: Brit. J. Surg. **19**:268, 1931. (*b*) Freund, E.: Arch. Surg. **28**:849, 1934. (*c*) McCune and Bruch.[2] (*d*) Albright and others.[3] (*e*) Goldhamer.[4]

7. (*a*) Imbert, L., and Huguet, J.: Gaz. méd. de France (supp. Radiol.) **44**: 191, 1937. (*b*) Marottoli, O. R.: An. de cir. de Rosario **3**:420, 1937. (*c*) Satanowsky, S.: Rev. ortop. y traumatol. **6**:287, 1937. (*d*) Pagniez, P.; Plichet, A., and Fauvet, J.: Bull. et mém. Soc. méd. d. hôp. de Paris **54**:733, 1938. (*e*) Robson, K., and Todd, J. W.: Lancet **1**:377, 1939. (*f*) Adams, C. O.; Compere, E. L., and Jerome, J.: Surg., Gynec. & Obst. **71**:22, 1940. (*g*) Elmslie, R. C.: Proc. Roy. Soc. Med. **27**:973, 1934. (*h*) Fairbank, H. A. T.: ibid. **27**: 977, 1934. Borak and Doll.[5]

of bone" has the advantage of being a short name and of separating the condition from other conditions with which it has been confused. Evidently others share our preference for this name, as, since 1938, six articles have appeared in which the condition has been described under that head.[8]

SURVEY OF CASES

Details of at least 90 cases of fibrous dysplasia of the skeleton (including the 15 new ones which we are adding in the present article) are now available. Of the 90 cases, 32 are accounted for in the literature to 1937. Most of these are reviewed in the papers by McCune and Bruch,[2] Albright and associates [3] and Lichtenstein,[1] who themselves add 1, 7 and 8 cases, respectively. To these 48 cases, the literature from 1937 on adds 27 (covered in sixteen articles). To these 75 cases we are now adding 15. This total of 90 certainly does not include all the cases, and even some of the graver ones have probably been overlooked because of the obscurity of the titles under which they have been described. Even so, it must be evident that fibrous dysplasia of bone is far from being a rare disease. On the contrary, it appears, by a wide margin, to represent the second most common systematized anomaly of skeletal development, being outranked only by multiple exostosis.

The sex of the patient was known in 86 of the 90 cases; in 51 the patient was a female, and in 35, a male.

The extent and the localization of the bone lesion are clearly known in regard to 87 of the 90 cases. In 15 of these 87, the disorder seemed to be restricted to one bone or part of a bone, i. e., to be monostotic.[9] In 17 of the 87, though more than one bone was affected, the skeletal involvement was still rather limited. Specifically, in 12 of these 17, only several bones of a single limb were affected; i. e., the involvement was monomelic. In the 5 others, it was still limited, two or three isolated bones being affected, irrespective of their relation to a limb. In another 29 of these 87 cases, the skeletal involvement was more extensive, but the lesions were still exclusively or at least predominantly one sided in

8. (*a*) Garlock, J. H.: Ann. Surg. **108**:347, 1938. (*b*) Case Histories of the New York Institute of Clinical Oral Pathology, Arch. Clin. Oral Path. **2**:374, 1938. (*c*) Horwitz, T., and Cantarow, A.: Arch. Int. Med. **64**:280, 1939. (*d*) Moehlig, R. C., and Schreiber, F.: Am. J. Roentgenol. **44**:17, 1940. (*e*) Denstad, T.: Acta radiol. **21**:143, 1940. (*f*) Stauffer, H. M.; Arbuckle, R. K., and Aegerter, E. E.: J. Bone & Joint Surg. **23**:323, 1941.

9. The number of cases of the monostotic form is certainly much larger than would appear from this. We made practically no attempt to sort them out from among the masses of cases discussed under such headings as "localized osteitis fibrosa," "localized fibrocystic disease of bone" and "osteodystrophia fibrosa localizata." Indeed, it would be difficult to sort them out, for a conglomeration of conditions has been described, usually inadequately, under these titles.

distribution, and, altogether, these cases can still reasonably be classified as representing moderate involvement. In the remaining 26 cases, very many bones were affected, though one side was still often more heavily implicated than the other, and these cases seem to belong in the category of severe involvement.[10]

Altogether, however, it seems to us that the general pattern of the disease so far as the gravity of the bone lesions is concerned can be properly conceived as embracing fewer cases showing extensive skeletal involvement and more of those showing involvement of only one or a few bones. In our opinion, the median severity is represented by cases showing lesions in a number of bones of one limb—more commonly a lower one—often with involvement of the corresponding side of the pelvis.

In only 32 of the 90 cases (about 35 per cent) was any degree of cutaneous pigmentation noted, although this is the commonest associated nonskeletal abnormality. Among these 32, males and females were about equally represented, and in the great majority of them the skeleton was severely affected. Only 20 of the 90 patients (about 22 per cent) exhibited indubitable clinical manifestations of endocrine dysfunction. With the single exception of a boy suffering from hyperthyroidism, the patients of this group were females (children or young adults) with a background of premature sexual maturation, and, in some instances, of hyperthyroidism and of premature skeletal growth and maturation as well. Also, in these 20 patients the skeletal involvement tended to be rather extensive.

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10. It seems worth while to give separately the data on the 23 cases which we studied, though of course these are included among the 90 just analyzed. In 9 cases of the 23, the condition appeared to be limited to a single bone and often, more particularly, to a portion of it. Specifically, in these cases, the single bone affected was a rib, a clavicle, a maxilla, a tibia or a femur. In 2 cases of the 23, though more than a single bone was affected, the involvement was restricted to portions of just a few isolated bones. Specifically, the calvarium, the radius and the contralateral humerus were affected in the first instance, and three ribs (not all on one side) and both pubic bones in the second. In each of 2 other cases of the 23, several long bones of a single lower limb were affected. In 8 others of the 23, the severity of the skeletal involvement was even greater, but in these cases, nevertheless, the distribution was still clearly predominantly unilateral. In 4 of them, one side of the pelvis and certain of the bones of the lower limb on the same side were involved together. In another, part of the skull on the same side was involved in addition, while in still another not only the skull but the contralateral humerus was also involved. In the remaining 2 of these 8 cases, certain bones of an upper limb and part of the skull on the same side were affected. Finally, in 2 of the 23 cases, the bone lesions were widely distributed, though still predominantly unilateral. In 1 of these 2, not only limb bones and the pelvis, but also some vertebrae, ribs and part of the skull were found affected.

If one reads only the case reports relating to the florid expression of the disorder, as seen particularly in infants or young children, one is likely to get an altogether exaggerated impression of the relative incidence and importance of these various associated extraskeletal aberrations. In our own series of 23 cases, in which the limited and moderately severe forms of the disorder are strongly represented but not the most extreme form, pigmentation of the skin was noted but twice, and only 2 of the female patients gave a history of onset of menstruation at a significantly early age.

THE SKELETAL CHANGES

Gross Observations.—We have been able to find, in the literature, references to 3 cases of fibrous dysplasia which have come to autopsy.[11] However, on account of the customary reluctance to examine bones at autopsies, the data relating to the skeleton in these cases are meager or at least not as ample as one would wish them to be. On the other hand, in the case reported by Telford,[12] in which a lower limb was disarticulated at the insistence of the patient, a good gross survey picture of the condition in several badly affected bones was obtained. However, even biopsy specimens from individual bone lesions yield much valuable information.

As noted, we have had such specimens in all of our 23 cases, and these specimens have ranged from mere curettings of abnormal medullary tissue to resected large portions of badly affected bones. A composite picture of the gross appearances can be built up on the basis of the findings in some of these cases. Thus, in a case of monostotic disease with a lesion 3 cm. in diameter in the midshaft of the tibia of a girl of 5, the curettings were composed of whitish and somewhat myxomatous connective tissue which, on the whole, was not gritty (figs. 3*A* and 8*A*). In another case of monostotic involvement, with expansion of the sternal 6 cm. of a clavicle in a man of 23, the specimens consisted of a piece of bulging cortex, which was found thinned and irregularly eroded on its endosteal surface, and also many pieces of firm, grayish white fibrous tissue removed from the interior of the bone in the affected area. This tissue was rather yellow-white and collagenous in appearance, and most of it was not at all gritty (fig. 3*B*). Several inches of a rib resected from a man about 35, again in a case of monostotic involvement, showed that this portion of the rib had undergone fusiform expansion. The cortex of the expanded area was less than 1 mm. in thickness and presented evidence of roughening and callus formation at

11. Coleman, M.: Brit. J. Surg. **26**:705, 1939. Albright and others.[3b] Adams and others.[7f]

12. Telford, E. D.: Brit. J. Surg. **18**:409, 1931.

the site of a pathologic fracture. The interior of the rib was found completely replaced by a grayish yellow tissue, which was rather rubbery and compressible (fig. 4 *B*) and which in the vicinity of the fracture presented dark red-brown hemorrhagic flecking.

In still another case of monostotic disease, with involvement of a rib (fig. 9 *A*), in a man of 44, there was found in the interior of the rib, surrounded by gritty fibrous tissue, a small cyst containing several cubic centimeters of straw-colored fluid. However, as noted, cystic softening of the abnormal medullary tissue is encountered in fibrous dysplasia only occasionally, and in this case it seemed to have followed on a course of preoperative roentgen therapy to the affected rib. Furthermore, in a case of monostotic disease (fig. 8 *C*) involving the neck and proximal portion of the shaft of a femur in a girl of 11, the specimen received showed that the cortical bone was everywhere attenuated, being no more than 1 or 2 mm. thick in some places. The outer surface of the cortex was smooth, but its inner surface appeared irregularly roughened and ridged. The interior of the bone was occupied by a grayish yellow rubbery fibrous tissue, but within the latter there were many small, circumscribed islands of hyaline cartilage measuring up to 1 cm. in diameter. Some of these cartilage islands appeared to be calcified peripherally (fig. 6 *B*). Furthermore, the fibrous tissue occupying the interior of the bone was grayish and collagenous in some places and yellowish and gritty in others, in accordance with the presence or the absence of metaplastic bone formation in the basic fibrous tissue.

The changes observed in the proximal half of a femur removed in the case of a boy of 11 on the mistaken premise that the lesion in it represented a malignant tumor proved to be entirely in harmony with those we have already described. However, in this case one could observe, in addition, the strong tendency of the condition to spare the epiphyses. Thus, the capital epiphysis of this femur did not show anything remarkable, and neither did the epiphyses of the greater and lesser trochanters. However, the neck appeared widened and foreshortened, and the proximal portion of the shaft was likewise appreciably expanded, so that it measured 4.5 cm. in its transverse dimension. The cortex was found markedly attenuated, especially on the lateral aspect of the femur. The capital epiphysis was composed of spongy bone, but the medullary region of the shaft distal to the epiphysial plate was everywhere occupied by a peculiar connective tissue. In some places, the latter was grayish white and essentially collagenous in character. Alternating with such areas were other fields which were more yellowish and gritty to the touch. In the region of the neck, this fibrous tissue showed red-brown streaks, indicating old hemorrhage. Within the fibrous tissue of the neck and shaft, furthermore, one could also discern a few small islands of hyaline

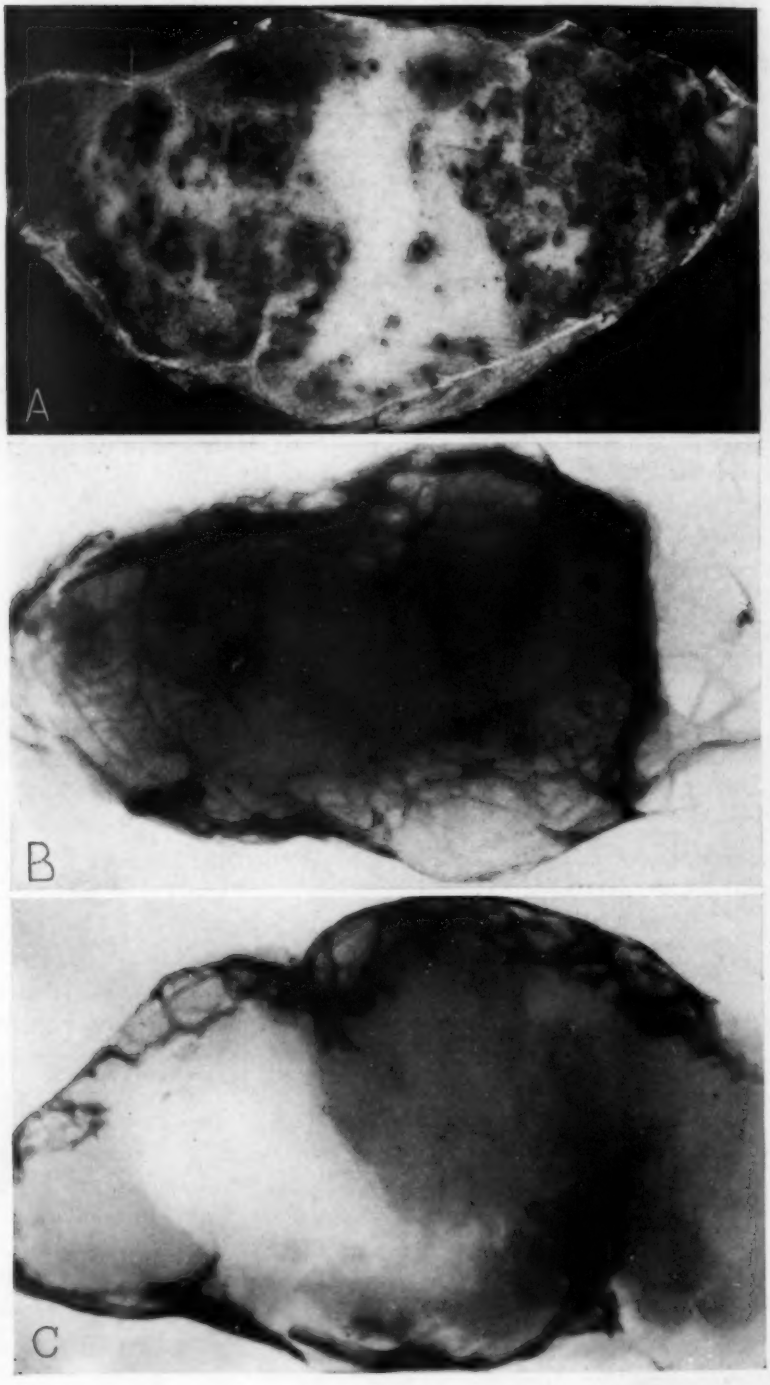

Figure 2

(See legend on opposite page)

cartilage. Indeed, the changes in this femur were entirely analogous in character to those in the femur of the amputation specimen described by Telford. In the latter, the cortical bone of the femur, over much of its extent, was reduced to a thin shell, and the medullary cavity of the bone was almost completely filled in by a white, tough, gritty fibrous tissue containing a few focal areas of hyaline cartilage.

Finally, 2 substantial specimens which we have examined in cases of polyostotic involvement are worth noting. One of these specimens was a segment of the left seventh rib of a woman of 35 in whom several other ribs were also affected (fig. 1). The resected portion of the rib (fig. 1 *A*) measured approximately 8 by 6 by 5 cm. in its greatest diameters. The expanded region (which abutted on the costal cartilage) was encased in a very thin bony cortex, which at no point was more than 2 mm. in thickness, and which near the end of the specimen was scarcely discernible except by its gritty feel. The periosteum appeared to have an outer fibrous layer, which could readily be peeled off, and a more delicate inner layer, which was intimately adherent to the thinned cortex. The lesion encased by this bony shell was composed of gritty fibrous tissue, which was rather rubbery in consistency and easily compressible. Its cut surface was fairly homogeneous in appearance but did show reddish and brownish flecks within the gray fibrous tissue. These apparently represented areas of congestion and blood extravasation. The fibrous tissue presented no areas whatsoever of cystic degeneration; nor were any islands of cartilage discernible within it. In some fields where appreciable ossification was in progress, the fibrous tissue felt distinctly

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EXPLANATION OF FIGURE 2

A, photograph showing upturned cut surface of the resected crest end of an affected ilium. Note the tremendous thickening of the latter. In this picture, mottling due to the presence of hemorrhages and dilated vascular spaces in the tough, rubbery tissue filling the interior of the expanded bone is particularly prominent. The specimen comes from a woman of 23 who presented involvement not only of the ilium but also of the femur and tibia on the same side (see fig. 10 *B*).

B, roentgenogram of the entire specimen, taken with the cut surface turned down onto the plate. Note the loculated or "soap-bubble" appearance of the shadow cast. That this appearance is not produced by septums running through the entire thickness of the specimen, but rather by mere ridges projecting inward from the convex surface is shown by *C*.

C, roentgenogram of a thin slab taken from the cut end of the specimen shown in *A*. Note that the loculations are limited to the periphery. Indeed, subsequent maceration of the specimen proved, as did that shown in figure 1 *C*, that it was merely the projection of many ridges from the inner surface of the cortex that was responsible for the multilocular appearance and that actually, as figure 2 *A* shows, the interior of the bone was solidly filled.

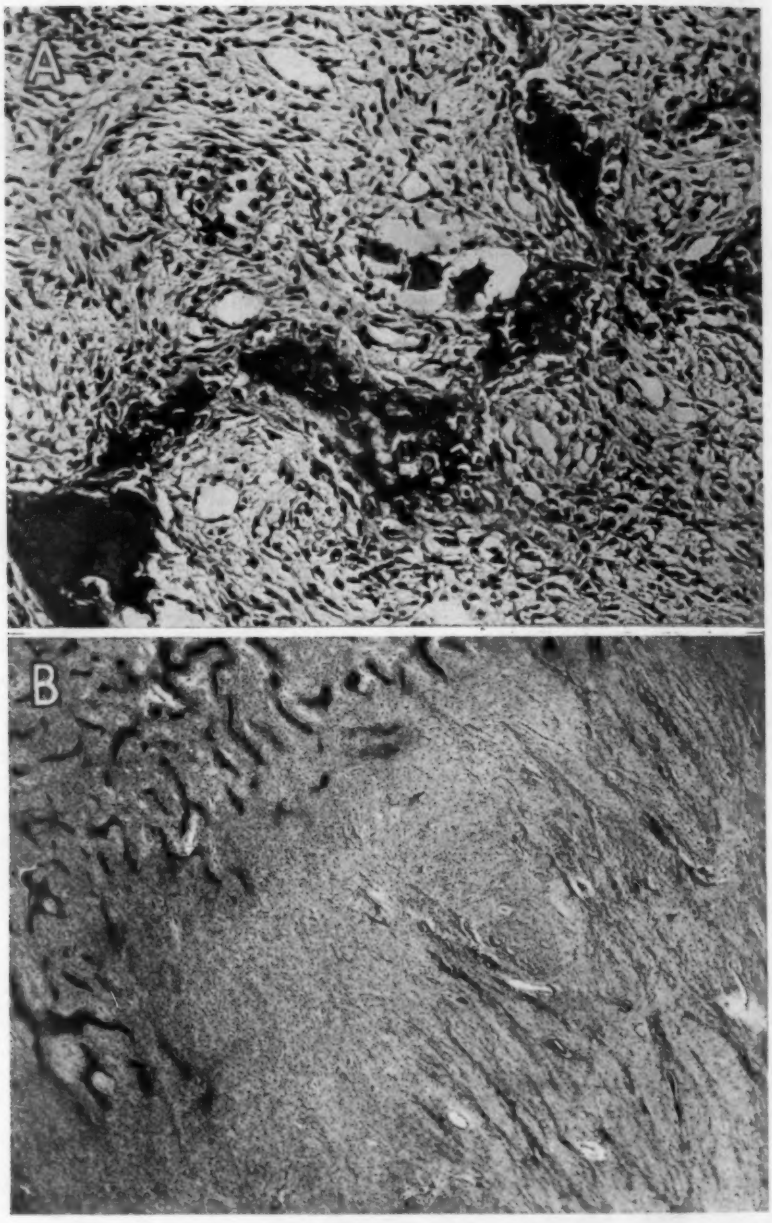

Fig. 3.—*A*, photomicrograph (× 200) showing the histologic character of the tissue which had completely filled the lesion pictured in figure 8 *A*. Note that the stroma is composed of small, slender spindle cells in rather loose and walled arrangement and that trabeculae of atypically calcified osseous tissue formed through metaplasia of the basic connective tissue are present.

B, photomicrograph (× 10) showing the histologic character of the tissue from a whitish field in the specimen shown in figure 2 *A*. Note that, except in the upper left hand corner, where appreciable ossification has occurred in the basic connective tissue, the latter is poorly cellular and distinctly collagenous.

gritty; in other fields, having comparatively little bone in them, it appeared more collagenous in character.

The other specimen represented a large segment of iliac bone from a woman of 23 years whose record was described in the earlier paper (case 3). In the roentgen picture (fig. 10 *B*) this ilium appeared enlarged and expanded and cast a rarefied and pseudotrabeculated "soap bubble" shadow. At the widest point of the specimen (fig. 2 *A*), the ilium had expanded to about six times its normal size. The cortex of the expanded ilium was extremely attenuated except at a few sites where it was reenforced on its inner surface by short bony spurs (fig. 2 *B* and *C*). The expanded ilium was solidly occupied by connective tissue, which was rather white and collagenous in some places and reddish and vascular in others, while in still others it showed areas of transition between these sites.

Microscopic Observations.—As noted, the interior of a given bone wherever it is affected is occupied by fibrous connective tissue often varying from place to place in its detailed histologic composition. Specifically, apart from the osseous or the cartilaginous elements it may contain, this connective tissue, in some places or throughout, may be rather cellular, composed of immature, small, slender spindle cells in rather loose and whorled arrangement (fig. 3 *A*). On the other hand, in some places or throughout, the connective tissue may be rather poorly cellular and highly collagenous (fig. 3 *B*). Furthermore, in some areas it may appear edematous or myxomatous or even show some cystic softening (fig. 7 *B*). Altogether, it would seem that the spindly and rather loosely whorled connective tissue is the basic connective tissue and that the collagenous, edematous or myxomatous areas represent modifications of this basic tissue. The connective tissue in the affected area is but meagerly interspersed with blood vessels, which are thin walled and of small caliber. On the whole, the more cellular, spindly areas are those richer in blood vessels.

In addition, the connective tissue in the affected area is likely to present trabeculae of bone formed through metaplasia. One finds little evidence of osteoclastic resorption of such trabeculae, and whatever reconstruction they undergo seems to proceed very slowly. In general, the more cellular and spindly areas are the more rich in osseous trabeculae, as in blood vessels. The trabeculae of metaplastic fiber bone are of variable size and contour. They are irregularly dispersed within the fibrous tissue, not following any regular pattern, and their number and location seem to be determined by the random distribution of the blood vessels in their immediate proximity. One may encounter whole fields showing scarcely any new bone deposition, and these are the areas which appear whitish and not especially gritty. On the other hand,

the fields containing a good many trabeculae of new bone are the areas which on gross inspection seem more yellow and distinctly gritty (figs. 3 *A* and *B*, 4 *A* and *B* and 5 *A* and *B*).

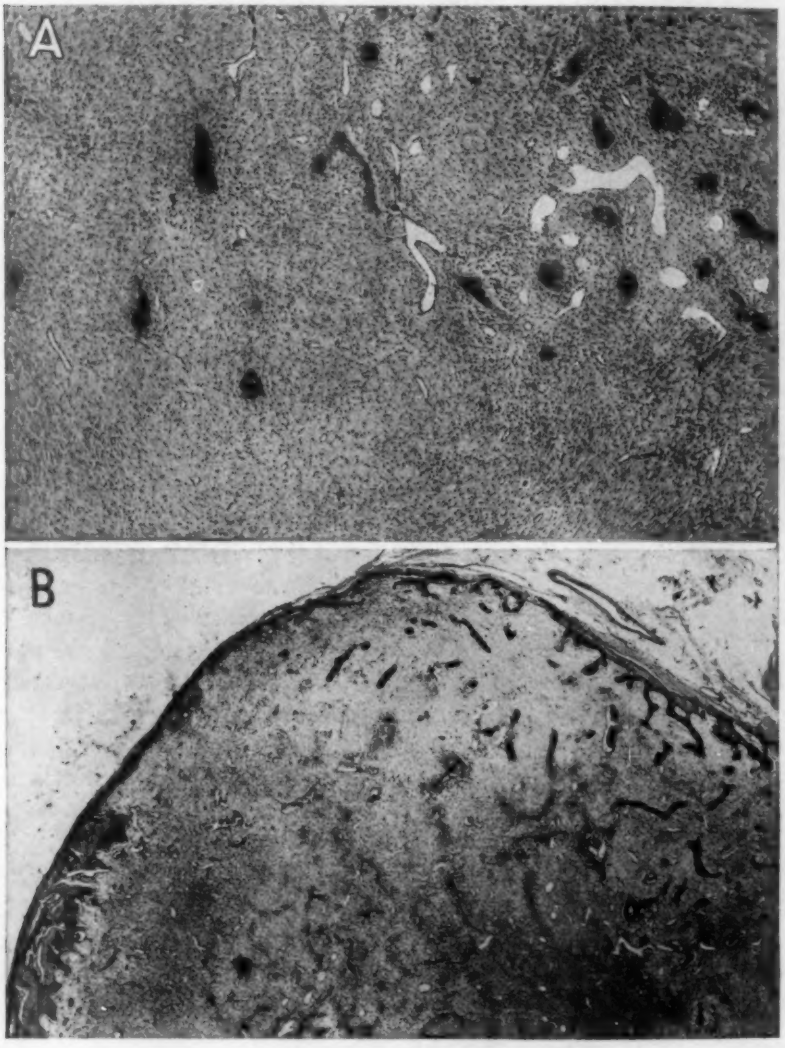

Fig. 4.—*A*, photomicrograph (× 45) showing the histologic character of tissue curetted from a solitary lesion in the sternal end of a clavicle. Note the comparatively sparse and small trabeculae of immature new bone dispersed in the poorly cellular and somewhat collagenous connective tissue.

B, photomicrograph (× 10) showing the histologic topography of a solitary lesion in a rib from a young man. What is pictured is part of a cross section of the affected rib. Note the thin and bulged cortex and the fibro-osseous character of the tissue filling the interior of the bone.

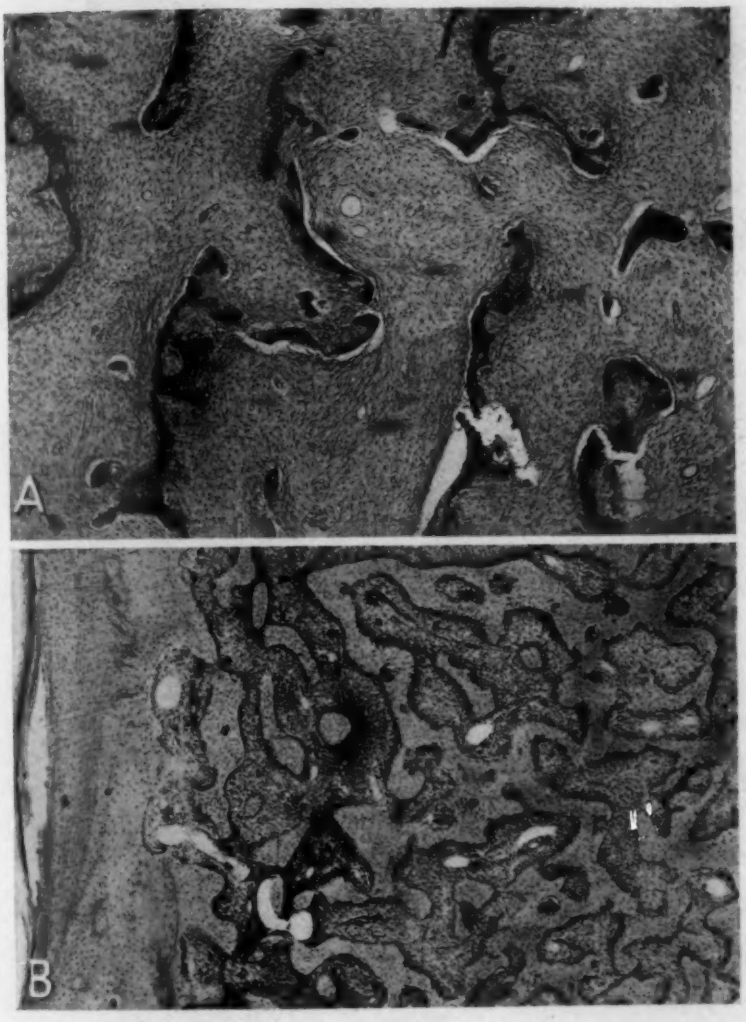

Fig. 5.—*A*, photomicrograph (× 20) showing the histologic pattern of the tissue which was curetted from the lesion pictured in figure 8 *B*. Note the spindle cell stromal tissue containing newly formed bony trabeculae, mainly in the proximity of blood vessels.

B, photomicrograph (× 20) showing the histologic pattern of the tissue in the biopsy specimen from the tibia pictured in figure 11 *B* and *C*. Note the thin cortex, which is undergoing erosion on its endosteal surface but which shows no obvious new bone apposition on its periosteal surface. The tissue occupying the interior of the bone shows numerous thick osseous trabeculae embedded in spindle cell connective tissue. These trabeculae were, however, not heavily ossified. Indeed, this medullary tissue, though hard and rubbery, could easily be cut with a knife.

Though the connective tissue on the whole tends to be relatively avascular, one sometimes encounters sporadic fields dominated by numerous enlarged and also engorged thin-walled blood channels (fig. 7*A*). Surrounding them, one may also observe blood extravasations. Indeed, such fields may show up even in the gross specimen as reddish zones of congestion or punctate hemorrhages (fig. 2*A*). The presence of hemosiderin pigment in such areas points to previous capillary hemorrhages which have been resorbed. Nests of multinuclear (giant) cells may be prominent in relation to such dilated and engorged blood vessels or to areas of blood extravasation. On the whole, these giant cells are considerably smaller than those of giant cell tumor and contain fewer nuclei—generally less than a dozen. They seem to represent merely multinuclear skeletal phagocytes, formed through coalescence and transformation of the stromal connective tissue cells.

Small islands of hyaline cartilage may be found embedded in the connective tissue in an occasional fibrous dysplastic lesion, and apparently these islands, too, result from metaplasia (fig. 6*A* and *B*). The cartilage foci are likely to be sporadic in their distribution, and they are generally rather small—considerably less than 1.0 cm. in diameter. In some instances, however, they are larger and more numerous and may even constitute a striking feature of the lesion. They tend to become calcified peripherally and eventually undergo some degree of endochondral ossification.

Although this fact has not been generally appreciated, the occurrence of such islands of hyaline cartilage within the lesion is not fortuitous. On the contrary, it undoubtedly represents an integral part of the dysplastic process. It is true that we have not incorporated that concept into the name chosen to designate the condition, but that is only because it would have made the name unduly cumbersome. This feature, moreover, points to a kinship between fibrous dysplasia of bone and skeletal enchondromatosis, which we likewise regard as a congenital systematized defect of development. These two conditions may be regarded as first cousins, so to speak.

The question of cyst formation within an occasional bone lesion deserves further comment, particularly in view of the tendency in some quarters to blanket all fibrous dysplastic lesions, even those not in the least cystic, under the term "fibrocystic disease of bone." In our earlier paper we stated that we had not encountered cysts in any of our specimens, even on microscopic examination. Since then, we have seen two lesions, one in a rib and another in an iliac bone, in which small fluid-containing cystic spaces were present within the fibrous tissue of the affected bones. In both instances it was possible to determine from examination of the wall of the cyst that the cystic area had developed

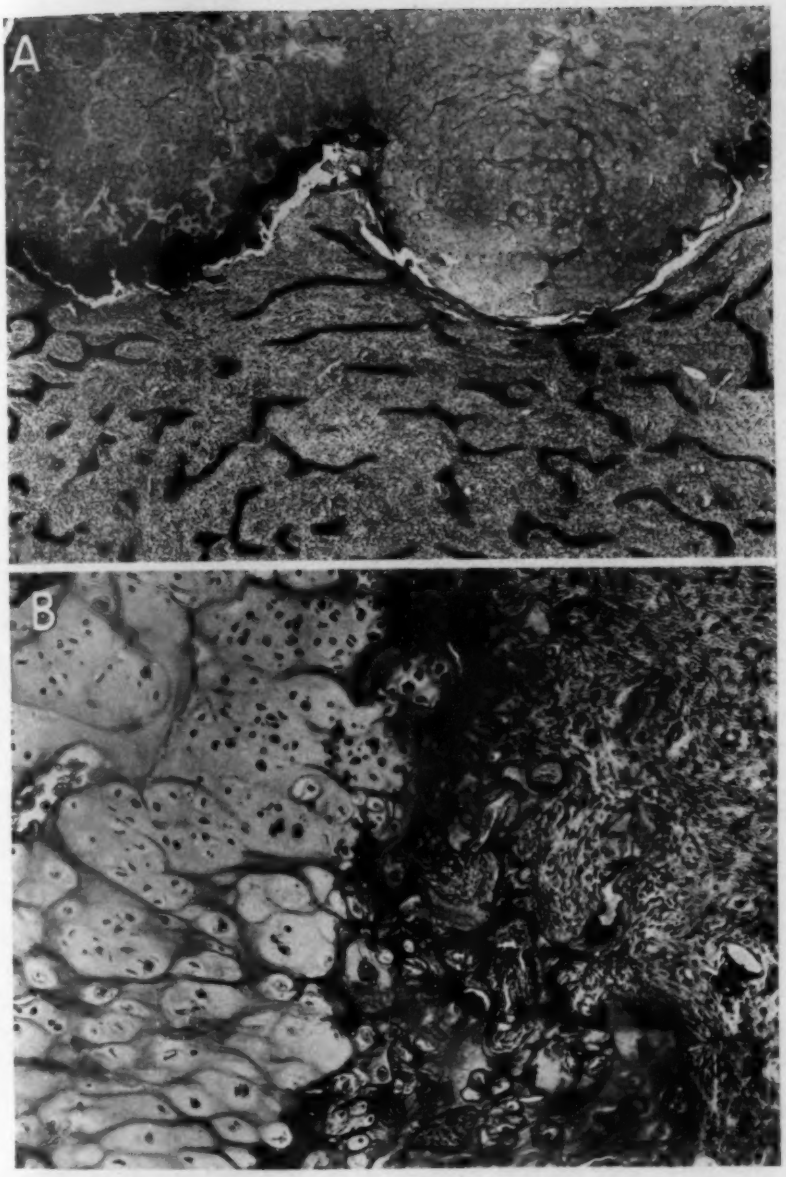

Fig. 6.—*A*, photomicrograph (× 15) showing the histologic structure of some tissue obtained by curettement of the lesion of the femur pictured in figure 10 *B*. Note, above, the mass of hyaline cartilage, with evidence of calcification at its periphery and, below, the dysplastic fibro-osseous tissue in which this cartilage was embedded.

B, photomicrograph (× 90) showing the histologic structure of some of the tissue curetted from the lesion pictured in figure 8 *C*. Many islands of hyaline cartilage were found embedded in the dysplastic fibrous tissue, a large quantity of which was curetted from the lesion in question. Note, to the left, the hyaline cartilage undergoing calcification and ossification where it is abutting on the stromal connective tissue.

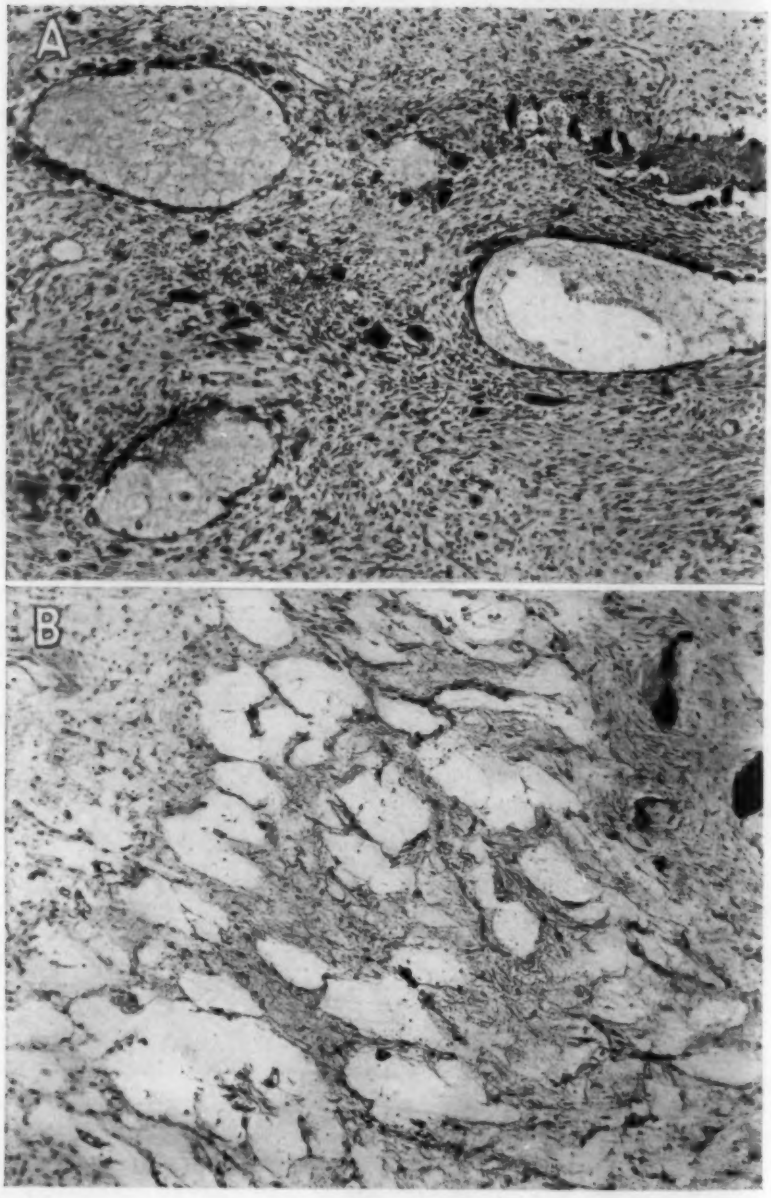

Figure 7

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secondarily as a result of softening of the connective tissue in the interior of the bone. Possibly a cyst may form where a hemorrhage has occurred somewhere within the lesion. Possibly, also, a cyst may develop in an area where collagenization of the connective tissue has interfered with the circulation through the lesion. Again, in 1 of our cases it appeared possible that roentgen treatment promoted cystic softening. Whatever their mode of development, these small foci of cystic degeneration that may be observed in an occasional instance of fibrous dysplasia are certainly of minor significance in the pathologic picture as a whole, and one is scarcely justified on their account in speaking of "fibrocystic disease."

Finally, one may find a few small lymphocytes or other mononuclear cells within the stroma of the lesion, mainly around blood vessels. They are not observed in every instance, and even when present, seem to have little significance. Even less frequently, one may encounter a few nests of foam cells within the fibrous tissue, just as one may find them in many other lesions of bone. The deposition of lipoid in such instances is clearly secondary and hardly represents a noteworthy feature of the condition, except so far as it may lead to a mistaken diagnosis of xanthomatosis.[12a]

Fibrous dysplasia taken as a whole, when one has once become familiar with its general appearance, is distinctive enough to be easily recognized. Indeed, a single glimpse of the character of the dysplastic connective tissue and of the primitive new bone within it, even in the absence of cartilage foci, will very often suffice to indicate the anatomic diagnosis.

12a. Snapper (Chinese M. J. **56**:303, 1939) and Landoff (Acta orthop. Scandinav. **11**:70, 1940) seem to have fallen into this error. Specifically, the observation of a few foam cells in a lesion of fibrous dysplasia led them to label cases of this condition as instances of lipoid granulomatosis or xanthomatosis.

EXPLANATION OF FIGURE 7

A, photomicrograph (× 75) showing the histologic pattern of tissue from a dark, mottled area of the specimen shown in figure 2 *A*. Note the dilated and thin-walled blood channels collared by multinuclear (giant) cells, and the blood extravasations in the stromal connective tissue, which is spindly in some places and collagenous in others.

B, photomicrograph (× 75) showing the histologic picture presented by some of the tissue curetted from the tibia in the same case as that from which came the ilium and the femur pictured in figure 10 *B*. Note that the stromal connective tissue is edematous and is beginning to undergo focal cystic degeneration. However, no gross or microscopic cysts were seen in the tissues removed from the ilium (fig. 2) and the femur (fig. 6 *A*).

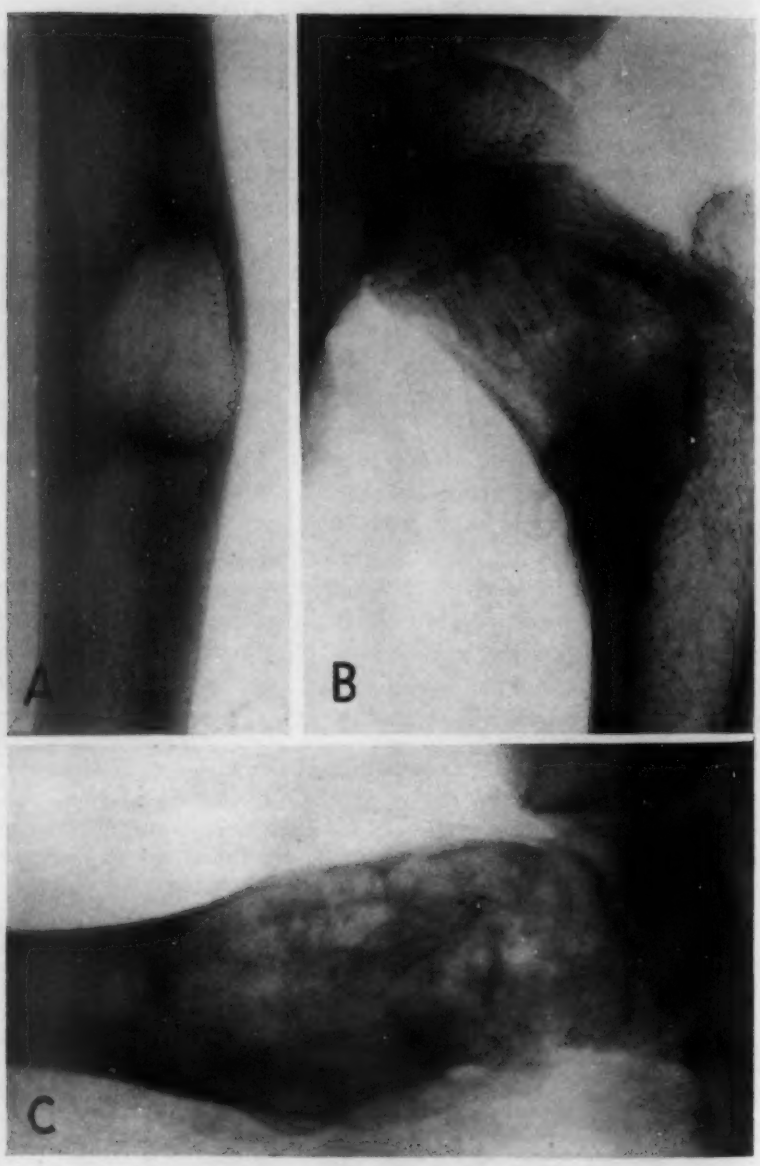

Figure 8

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CLINICAL EXPRESSIONS OF THE DISORDER

Cases Presenting Monostotic Involvement.—As noted, far from being unusual, the monostotic expression of fibrous dysplasia seems really to be more common than the florid expression at the other extreme of the range of skeletal involvement. As already stated, our own experience with this form of the disease includes 9 cases, involving mainly older children, adolescents and young adults. In these cases, the single bone affected was a rib, a clavicle, a maxilla, a tibia or a femur. In some instances, the lesion was rather small in relation to the size of the bone, while in others a substantial part of the bone was affected. Typical examples of monostotic involvement have also been described by Elmslie [12] and Freund and Meffert.[13]

The clinical complaints in these 9 cases were rather mild on the whole, depending more on what bone was affected than on the extent of its involvement. Thus in a case in which a rather large lesion of a rib was present, the lesion was discovered only incidentally, by fluoroscopy, in the course of a routine physical examination. Again in a case in which a rib was involved, the lesion was entirely asymptomatic until a pathologic fracture had occurred. On the other hand, in a case with involvement of the neck and the adjacent shaft area of a femur, the patient, 11 years old, gave a history of limp and intermittent pain of five years' standing. Especially when a bone of very superficial location, such as a maxilla, a clavicle or a rib is involved, a local swelling can often be palpated, irrespective of whether or not the condition is

13. Freund, E., and Meffert, C. B.: Surg., Gynec. & Obst. **62**:541, 1936.

EXPLANATION OF FIGURE 8

A, roentgenogram of a solitary focus of fibrous dysplasia in the shaft of a tibia. Note the roundish rarefaction shadow and the localized expansion and thinning of the cortex. The area in question was filled by only very slightly gritty connective tissue whose histologic character is pictured in figure 3 *A*. The patient was a child of 5, and the lesion was asymptomatic until an infraction had occurred at its site.

B, roentgenogram of a solitary focus of fibrous dysplasia in the neck of a femur. Note the semicircular striated shadow there. The area in question was completely filled with gritty connective tissue, whose histologic character is pictured in figure 5 *A*. The patient was a boy of 13, whose lesion was discovered when he complained of slight pain in the hip.

C, roentgenogram of a solitary focus of fibrous dysplasia in the neck and adjacent portion of the shaft of a femur. It can be seen that the head of the bone is not affected, and other views show that the greater and the lesser trochanters were also spared. Note that in the affected area the bone is somewhat expanded, hazy and stippled. The area in question was filled with fibro-osseous tissue containing large islands of hyaline cartilage, as pictured in figure 6 *B*.

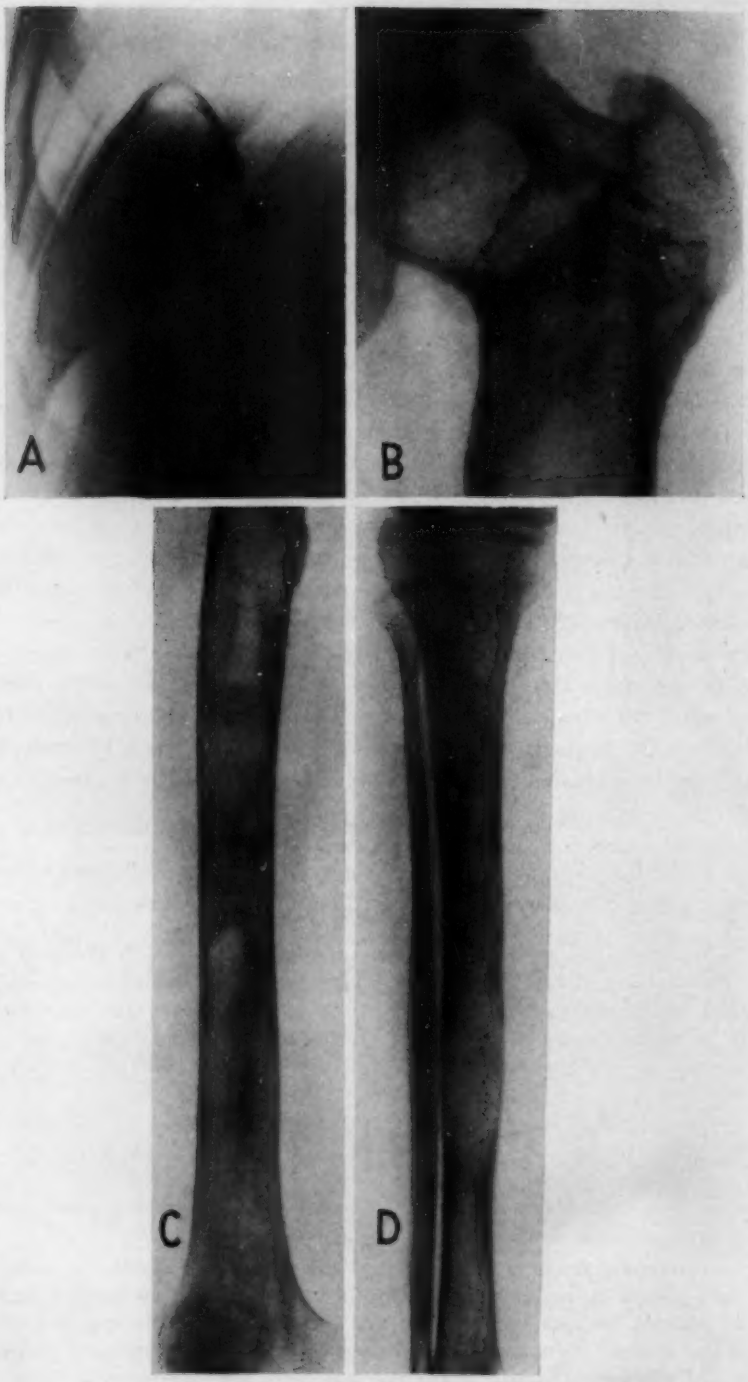

Figure 9

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creating clinical difficulties. Furthermore, in connection with these monostotic cases it is important to note that the lesion of bone is not associated with extraskeletal abnormalities and not even with patchy pigmentation of the skin.

On the basis of the roentgenographic findings, the condition in the monostotic cases is often difficult to identify. For instance, a smallish lesion in a shaft of a long bone may appear simply as a roundish or an oval area of rarefaction outlined by a cortex showing thinning from the medullary side and perhaps distention in part or throughout (fig. 8 *A*). Such a roentgen picture could reasonably be interpreted as representing a solitary unicameral bone cyst, but surgical intervention would reveal not a fluid-filled cavity in the bone but, instead, a focus of gritty fibrous tissue. A strikingly different picture is presented in 1 of the cases in which the lesion was in the neck of a femur. The lesion appeared roentgenographically as a large, clearly delimited semicircular shadow in which the base represented the inner cortex of the neck and which showed parallel striations running close together (fig. 8 *B*). A different appearance, again, was presented by another femoral lesion—this one not limited to the neck but involving also the proximal portion of the shaft. In this case, the neck of the bone was widened, and the entire affected area presented a cloudy, stippled and mottled appearance which could reasonably lead to interpretation of the lesion as an enchondroma (fig. 8 *C*). A somewhat similar roentgenographic appearance was presented by a lesion occupying the sternal third of a clavicle. In the case of a solitary lesion of a rib, the roentgen picture revealed an irregular expansion in the shape of a crooked sweet potato, about 5 inches (12.5 cm.) in its long axis. The cortex of the expanded rib appeared considerably thinned, and the rarefaction shadow of the disease focus itself was traversed by faint trabecular markings (fig. 9 *A*).

Perhaps we have now described enough solitary lesions to show how widely the roentgen picture may differ from case to case of this kind.

EXPLANATION OF FIGURE 9

A, roentgenogram showing a solitary focus of fibrous dysplasia in a rib. The patient was a man of 44 who had no symptoms referable to the lesion, the latter being discovered incidentally in the course of a routine check-up physical examination.

B, roentgenogram showing involvement of the neck and upper portion of the shaft in a case of polyostotic fibrous dysplasia involving the long tubular bones of a lower limb. The rest of the femur and the tibia and the fibula are pictured in *C* and *D*. The patient, a girl of 19, presented no extraskeletal abnormalities.

C and *D*, roentgenogram showing involvement of the remaining portion of the femur and of the tibia in fibrous dysplasia, in the case mentioned in connection with *B*.

It should be apparent also why these lesions can be mistaken roentgenographically for bone cyst, enchondroma or even giant cell tumor. Thus, for a definitive diagnosis, a biopsy is necessary. Even when a biopsy has been performed, a pertinent case may still remain buried if it has been classified, on the basis of the anatomic findings, as one of localized osteitis fibrosa, osteodystrophy or fibrocystic disease.

Cases Presenting Involvement Limited to a Few Bones.—As noted, there are cases in which several bones of a single limb or several bones regardless of their relation to a limb are affected. In these cases, too, nonskeletal abnormalities are generally absent, and any clinical complaints relate to the skeleton alone. In part, such complaints depend on what particular bones are affected and the severity of their involvement. In addition, it is to be noted that the earlier in life the skeletal lesions become pronounced, the more likely it is that they will give rise to clinical complaints. Thus, a child or an adolescent person in whom some bones of a lower limb are affected may have pain in a hip, limp, or even bowing deformity, and may also give a history of pathologic fractures, perhaps dating back some years. On the other hand, in some cases with involvement limited to one limb or to a few scattered bones, the condition may be present for years and even well into adult life before its discovery. This may come about through the ultimate appearance of some clinical complaint, less often incidentally in the course of a general physical examination, or perhaps only at autopsy.

Our own records include 2 instances of involvement limited to several bones of a lower limb (monomelic involvement) in which the disease was discovered during adolescence (figs. 9 *B, C* and *D*). Denstad [8e] has described a case of this kind—that of a young woman of 18 who sustained repeated spontaneous fractures from the age of 7 on and who had complained, furthermore, of pain in the thigh and a limp. She showed involvement of the left femur, tibia and fibula, but no other stigmas of the disease. The literature contains many other references to the monomelic type of the disorder, and in particular cases have been described by Hunter and Turnbull,[6a] Elmslie,[7g] Freund and Meffert,[13] Hodges, Phemister and Brunschwig [14] and Adams, Compere and Jerome.[7f] Cases of monomelic involvement have also been described by Imbert and Huguet,[7a] Marottoli [7b] and Satanowsky,[7c] albeit they interpreted their cases as representing limited or monomelic Recklinghausen's disease of bone.

Our records also include 2 cases of fibrous dysplasia in a few scattered bones, in which the disease was not discovered until the subject was well

14. Hodges, P. C.; Phemister, D. B., and Brunschwig, A.: The Roentgen-Ray Diagnosis of Diseases of the Bones and Joints, New York, Thomas Nelson & Sons, 1938, p. 152.

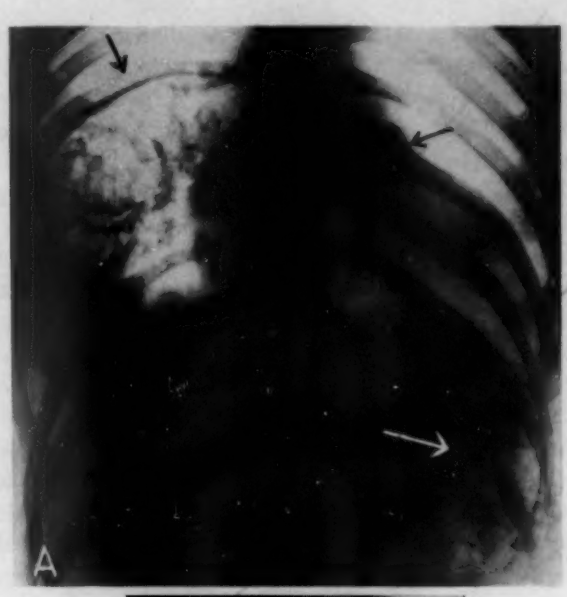

Fig. 10.—*A*, fibrous dysplasia of a number of ribs. The patient was a woman of 35. One of the affected ribs was resected, and the lesion in question is pictured in figure 1.

B, roentgenogram showing involvement of the ilium and the femur. The case is one in which the tibia was also affected. The patient was a girl of 23 who presented no extraskeletal abnormalities. Note the expansion and the rarefied, trabeculated appearance of the iliac bone, the coxa vara and the clublike expansion of the upper part of the femur. Pathologic material from the ilium, the femur and the tibia in this case are pictured in figures 2, 6 *A* and 7 *B*, respectively.

on in adult life. In 1 case, the patient, a woman of 35, had been complaining, but only recently, of severe intercostal nerve pains, and these were found to have resulted from tumor-like expansion of a number of ribs (fig. 10 *A*). Incidentally, the roentgen films of this patient's pelvis showed that the pubic bones were apparently also involved. The other patient was a woman of 40 who never experienced any skeletal complaints until about three years prior to observation, when she noticed that one forearm was becoming deformed. She also had calvarial changes, and while she was under observation a lesion appeared in the other arm as well.

When one encounters roentgenographically a lesion suspected of representing fibrous dysplasia, a search for additional osseous lesions is indicated. Certainly, if one finds such a lesion in a limb bone, one should investigate at least the rest of the limb, probably the adjacent part of the trunk (i. e., the pelvis or the shoulder region on the side of the lesion) and possibly also the skull. Should additional lesions be found in these skeletal areas, the rest of the skeleton should also be roentgenographed. If one follows this procedure, the likelihood of missing the polyostotic aspect of a case will be small, while at the same time unnecessary roentgenography will be reduced to a minimum.

In general, what has been said about the roentgen picture presented by the affected bone when only one is involved applies also to the individual affected bones in the polyostotic form of the disorder. It is true that the appearance presented by any individual affected bone in the latter cases may not be sufficiently distinctive to permit diagnosis of the condition. However, should several bones of a single limb be affected, with or without adjacent trunk bones, the diagnosis of fibrous dysplasia ought to suggest itself promptly, though the possibility of the limited form of skeletal enchondromatosis (Ollier's disease) should also be considered. Indeed, cases with very extensive skeletal involvement are even easier to diagnose roentgenographically, though those who are not familiar with fibrous dysplasia rather often misinterpret them as instances of hyperparathyroidism.

An individual bone considerably involved in fibrous dysplasia presents roentgenographically a number of discrete rarefactions, or, if the latter have become confluent, the major part of the bone may appear more or less diffusely rarefied (figs. 9 *C*, 10 *A* and *B* and 11 *B* and *C*). The rarefactions reflect the replacement, in the affected area, of the spongy bone and of the adjacent inner surface of the cortex by fibrous connective tissue, which, of course, is relatively radiolucent. If within this tissue there has been substantial metaplastic ossification, the rarefaction shadow is likely to present a mottled or rather cloudy ground glass appearance. The latter expresses the character of the immature new bone. In some lesions one may even note stippling here and there, indicating the pres-

ence of islands of ossifying cartilage. On the other hand, in areas in which the fibrous tissue filling the interior of the bone is not undergoing appreciable metaplastic ossification and in which there are no islands of cartilage also undergoing ossification, the rarefaction shadow resembles that which would be cast by a cyst.

The cortex in the affected area is usually found thinned and may even have been reduced to a mere shell in consequence of erosion of its endosteal surface by the dysplastic fibrous tissue. We have never seen, however, an instance in which the latter has perforated the cortex and extended into the parosteal tissue. Except in the region of a fracture, the outer surface of the thinned cortex shows no periosteal new bone apposition. Indeed, any new, reenforcing periosteal bone being laid down is deposited so slowly that roentgenographic evidence of it is generally lacking. On the other hand, there is no appreciable resorption of the outer surface of the cortex, and there is never any evidence of scalloped erosion beneath the periosteum, as seen in hyperparathyroidism.

At the site of a recent pathologic fracture one generally sees considerable cortical thickening, resulting from organized callus. Old infraction lines stand out as relatively opaque linear markings, usually more or less at right angles to the cortex. Affected flat bones, in particular, may present a "trabeculated" appearance (figs. 1 *B*, 10 *A*, 2 *B* and 10 *B*), but actually this merely reflects the presence of short bony spurs and low ridges on the endosteal contour of the thin, eroded cortex (fig. 1 *C*). These bony spurs and ridges develop apparently as a reaction to the slow erosion and are present on the entire circumference of the lesion. Actually, they extend but a short distance into the fibrous tissue, but when projected on the flat surface of a roentgen film they seem to traverse the whole lesion, giving the latter a loculated appearance.

As to contour, an affected bone may remain normal, but often it is more or less expanded instead. The tendency to expansion is not surprising, since the thinned cortex of the affected bone site offers but little resistance to the growing fibrous tissue which it encases. We have examined a substantial portion of an iliac bone which was expanded to fully six times its customary thickness (fig. 2 *A*). An affected rib (fig. 1 *A*) or an affected calvarium (fig. 11 *A*) or facial bone may be found expanded into an obvious tumor-like mass. In severely affected long bones, one may observe not only expansion but also more or less concomitant deformity, especially of those bones subject to weight bearing. Involved femurs, for instance, may be sufficiently deformed by coxa vara and anterior and lateral bowing to present a jug handle or a question mark appearance. Such deformed bones may also become more or less shortened, although, as a rule, the limb shortening is not nearly as pronounced as in skeletal enchondromatosis.

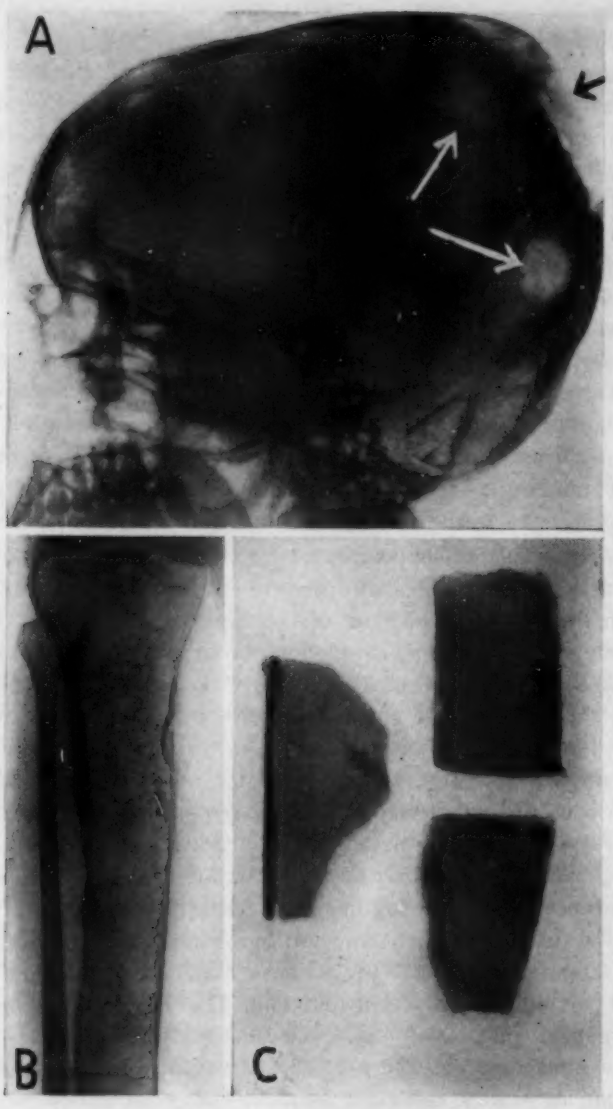

Figure 11

(See legend on opposite page)

Cases Presenting Moderate Involvement.—It may be somewhat arbitrary to make a distinction between limited and moderate involvement, but on the whole this distinction appears useful. We are placing in the "moderate" category the cases with involvement of more than just some bones of a single limb but not much involvement of the rest of the skeleton. In these cases, whatever other bones (such as pelvic bones, ribs or skull bones) are affected are likely to be on the same side of the body as the affected limb bones; i. e., the distribution of the bone lesions as a whole tends to be exclusively or at least predominantly one sided. Furthermore, an occasional patient of either sex presenting the moderate form of skeletal involvement will exhibit also one or more yellow-brown pigment patches on the back, buttocks or elsewhere. An occasional female patient, moreover, may give a history of catamenia at an unusually early age. More often than not, however, such extraskeletal disturbances will be entirely lacking.

Our material includes 4 cases in which only the pelvis and some lower limb bones on the same side were affected. In 2 other cases, skull bones on the same side were also involved, and in 1 of these 2 the contralateral humerus likewise. In 2 additional cases, the involvement was restricted mainly to some bones of an upper limb and bones of the skull on the same side. Furthermore, the recent literature on the condition in question contains many references to similar cases, including the reports by Moehlig and Schreiber [8d] and Adams and associates [7f] and the report in the *Archives of Clinical Oral Pathology*.[8b] The case recorded by Horwitz and Cantarow [8c] may also be put in the category of moderate involvement. However, in that case the lesions were present throughout the pelvis and in the bones of both lower limbs, including some of the foot bones.

EXPLANATION OF FIGURE 11

A, roentgenogram of a skull showing fibrous dysplasia. Note the rarefied defects in the calvarium and the large blister-like expansion of the outer table posteriorly. The patient, a woman of 22, showed, in addition, involvement of a number of vertebrae and ribs, the right side of the pelvis, the left pubic bone, the right femur and the right tibia. Her complaints began at the age of 5 but she presented no extraskeletal abnormalities.

B, roentgenogram of the tibia in the case mentioned in *A*. Note the paper-like thinness of the cortex and the hazy character of the shadow cast by the tissue filling the interior of the bone.

C, roentgenogram of biopsy specimen taken from the tibia shown in *B*. The histologic pattern of the tissue prepared from this specimen is pictured in figure 5 *B*. The hazy shadow cast by the slices of tissue shown, on the right hand side of the picture especially, reflects the presence of numerous but poorly ossified trabeculae of bone within the dysplastic connective tissue, as is evident in figure 5 *B*.

Most of the subjects presenting the disorder in its moderate form have clinical difficulties which they date back to childhood or adolescence, at least. In the cases in which bones of a lower limb are affected, there may be pain in the hip, limp or deformity, and the patients may have suffered from one or more pathologic fractures through affected bones. In the cases in which bones of an upper limb are affected, there may likewise be a history of pathologic fracture of one or another of the bones involved, with or without deformation. In cases in which the skull is involved in addition, there may be obvious swelling of some of the facial bones and even expansion of some part of the calvarium.

However, an occasional case may go unrecognized well into adult life, and be discovered only incidentally. Through the courtesy of Dr. A. Kenin, we were recently enabled to study such a case. The patient was a woman of 32 who came under medical care for a urinary complaint. In the course of roentgen examination of her abdomen, it was discovered that the right ilium and the upper portion of the femur were expanded and rarefied. Additional roentgen pictures were then taken, and they disclosed involvement of the rest of the femur and of the tibia and the fibula of the same limb. On being questioned, this patient did state that she had long been aware of a slight limitation of abduction of the right thigh, but it had never been associated with any pain and indeed interfered in no way with her activities, so that she had paid no attention to it. In this case, we examined biopsy specimens from the ilium and the tibia which confirmed the diagnosis.

Adams and associates also reported a case in point. Their patient was a man of 59 whose skeletal disease was completely asymptomatic and indeed had gone unrecognized until a roentgen film of the abdomen, taken in connection with a gastric complaint, had disclosed rarefaction shadows within the right ilium and the upper part of the femur. Necropsy shortly thereafter revealed the changes of fibrous dysplasia throughout the femur (except for the epiphysial regions of the head and the greater trochanter), in the ipsilateral tibia and fibula as well, and even in a rib where it had not been visualized roentgenographically.

As to the roentgenographic findings in these cases, what was said in the discussion of the cases representing the limited form of involvement is applicable so far as the individual bones affected are concerned, so that the reader has only to refer back to that discussion. It is also applicable to the grave cases, now to be described.

Cases Presenting Severe Involvement.—We are placing in this category the cases in which a major or at least a substantial part of the skeleton is found affected. Bones of two, three or even all four limbs may be involved, and all combinations of upper and lower limb implication have been observed. The other parts of the skeleton also tend to be

affected. In addition, in the cases of severe involvement the individual affected bones tend to be more extensively and seriously damaged than they are in the limited or the moderate form of the condition. Furthermore, the skeletal lesions in the severe forms of the disease are often so widely scattered that one can no longer speak of unilateral predominance. Nevertheless, some even among the cases of severe involvement still show the tendency to predominance of the involvement on one side of the body which has been noted in the cases showing limited or moderate involvement. Finally, it is in the cases with severe skeletal involvement that associated extraskeletal abnormalities are observed with some degree of frequency.

In the severe form of the disease, the condition usually manifests itself early in life, i. e., in childhood or even in infancy. Moreover, the skeletal involvement often leads to serious deformities and crippling disability. Affected limb bones are frequently found considerably expanded, bowed, shortened and otherwise deformed. Furthermore, such limb bones are rendered susceptible to repeated spontaneous fractures. As a result of the implication of a lower limb, disturbance in gait is likely to appear because of coxa vara and bowing of the femurs, so that waddling may be observed early in childhood as one of the first signs of the disorder. Extensive involvement of the innominate bones may be followed by deformity of the pelvic girdle. Substantially involved vertebral bodies may become partially collapsed and angulated, so that various degrees of kyphoscoliosis develop. Lesions of ribs may present as localized tumor-like expansions, and such areas too are prone to pathologic fracture. In the calvarium, a focus of fibrous dysplasia may bring about a localized expansion of the outer table, which can become quite prominent. In addition to calvarial changes and deformity of facial bones, skull plates in these cases often reveal striking thickening and opacity of the bones comprising the base of the skull, especially around the pituitary fossa.

Our own material includes 2 cases with the severe form of skeletal involvement. The literature contains reports of many additional cases of this character, and the reader can find summaries of most of the reports before 1937 in the papers by McCune and Bruch,[2] Albright and associates[3] and Lichtenstein.[1] We shall therefore consider here only the more recent ones which are of particular interest in this connection.

Pagniez, Plichet and Fauvet[7d] described the case of a young man of 26 with a history of several spontaneous fractures of long bones, the first of which occurred at the age of 3. Roentgen examination of the skeleton disclosed many osseous lesions which, though widely distributed in the upper and lower limbs, the pelvis, the vertebral column and the skull, were still predominantly right sided. The lesions in the calvarium

were described as being "pagetoid," and other commentators, too, have mentioned the "pagetoid" skull. The patient in question also presented several large brownish pigment blotches on his skin.

Coleman [11] published a case report which is particularly illuminating. The patient was a 13 year old boy with a history of repeated pathologic fractures starting at the age of 10. Roentgen examination of the skeleton revealed involvement of the facial bones (asymmetry), both humeri, the right pubis, both femurs and the right tibia. No significant pigmentation of skin was present, it is true. This case came to autopsy, and there were a number of significant extraskeletal findings, which will be mentioned in a later section.

The majority of the other recent reports on cases of particularly severe involvement have dealt with comparatively young female subjects who exhibited also some degree of cutaneous pigmentation and evidences of, or at least a history of, premature sexual maturation. The relatively large number of such reports is explainable by the striking character of the manifestations in these cases. Mondor and associates [15] have described a case of this kind. Their patient was a girl who presented extensive yet predominantly unilateral fibrous dysplasia of the skeleton and pigmentation of the skin, present since birth. Catamenia started at the age of 7, at about which time the secondary sex characteristics, notably the appearance of pubic and axillary hair, were also observed.

In the case described by Shallard,[16] the patient was an 18 year old girl with scoliosis, bowing of the right arm and leg and a history of several spontaneous fractures. Conspicuous blotchy pigmentation had been noted since the age of 1½, and the appearance of secondary sex characteristics at the age of 8. The patient also had a history indicating premature skeletal growth and maturation. The roentgen examination of the skeleton in this case revealed extensive bilateral fibrous dysplasia. The osseous changes were particularly marked in the bones comprising the base of the skull. Incidentally, this patient at one time was thought to have had hyperparathyroidism and was operated on, but no lesion from parathyroidism was found. Diez [17] has reported a similar case. His patient was an 18 year old girl with extensive bilateral fibrous dysplasia, blotchy pigmentation of skin and a history of menstruation and genital enlargement at the age of 5. She had sustained repeated pathologic fractures in childhood. When she was 12, her parathyroids were explored because hyperparathyroidism was suspected, but again no lesion due to parathyroidism was found.

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15. Mondor, H.; Ducroquet, R.; Leger, L., and Lawrence, G.: J. de chir. **53**: 593, 1939.

16. Shallard, B. T.: M. J. Australia **1**:558, 1940.

17. Diez, J.: Prensa méd. argent. **26**:1870, 1939.

Robson and Todd,[7e] too, have described a case of this same general character. Their patient was a woman of 33 who gave a history of many spontaneous fractures of limb bones starting at the age of 7 and of enlargement of the breasts and appearance of pubic hair at the age of 6, followed a year later by the onset of menstruation. It is interesting to note that the menstrual flow in the beginning was often excessive and unpredictable and that a fairly regular cycle was not established until more nearly the usual age of puberty. Although this patient's bone lesions were predominantly one sided, she too was subjected to a search for a parathyroid tumor.

Braid [18] described 2 cases in which practically the entire skeleton, including most of the limb bones, was affected and the presence of the condition was already evident in infancy. In the first instance, the patient was a boy who exhibited blotchy pigmentation of the face, neck and shoulders at the age of 4 months and who sustained multiple fractures of his limb bones starting at the age of 2. In the second case, the patient was a girl with a history of pigmentation of the face and back, first noted at the age of 3 months, of repeated fractures, the first of which developed at the age of 1½, and of enlargement of the breasts and onset of menstruation at 2½ years of age. Both of Braid's patients, furthermore, had survived icterus gravis neonatorum.

Summerfeldt and Brown [19] have also reported 2 pertinent cases of unusual interest. The patients were girls, 10 and 6 years old, respectively. Each had extensive fibrous dysplasia of the skeleton involving all four limbs and the skull, pigmentation of skin and manifestations of premature sexual maturation. In the first of these cases, a search for a parathyroid tumor had been made, with negative results. The second case is noteworthy in that, in addition, the child had had jaundice, present at birth and lasting four months, and had begun to menstruate at the age of 2. The cycle was very irregular, however, the periods occurring from three to six months apart, and the flow, though prolonged for two weeks, was scanty and painful. The sexual precocity seemed to be accompanied, moreover, by premature growth and by evidence of hyperthyroidism.

FURTHER CONSIDERATION OF THE NONSKELETAL ASPECTS OF THE DISEASE

The case histories cited in the foregoing section to illustrate the severe form of fibrous dysplasia show how commonly various nonskeletal changes appear in connection with this form of the disease. These cases only reemphasize what some of the earlier writers had already pointed

18. Braid, F.: Arch. Dis. Childhood **14**:181, 1939.
19. Summerfeldt, P., and Brown, A.: Am. J. Dis. Child. **57**:90, 1939.

out, namely, that in certain cases in which skeletal manifestations had appeared very early in life the patient may also show patchy pigmentation of the skin and pubertas praecox. For instance, Goldhamer [4] and Borak and Doll [5] had already stressed this fact in 1934. McCune and Bruch,[2] in their paper of 1937, shed special light on the nonskeletal aspects of the disorder in their discussion of a case of their own with not only precocious puberty and hyperpigmentation of the skin but, interestingly enough, hyperthyroidism as well. Albright and associates,[3] in their papers of 1937 and 1938, likewise called attention to the extraskeletal aspects of the disorder. Specifically, the 7 patients on whom they report all showed a few or even many brownish pigment blotches on the skin, and the 3 females among them gave, in addition, a history of premature sexual maturation. Since 1938, a number of similar cases have also been reported, and certain of them have been cited in foregoing paragraphs.

We shall now analyze the data on the different types of extraskeletal change as exemplified in the 90 collected cases (including the 15 new ones being added in the present discussion).

Pigmentation of Skin.—Among the 86 (of 90) patients whose sex was known there were 35 males, of whom only 15 showed any degree of abnormal pigmentation. Of these 15, 7 had numerous or very large brown pigment blotches, and 8 presented either only a large single patch or else a few yellow-brown spots or small patches which could be missed if one did not search for them. Of the 51 female patients, only 17 exhibited pigmentation, but, on the whole, when pigmentation was present in them it was quite likely to be conspicuous.

The pigment blotches may be observed anywhere on the skin, but they seem to occur most often on the scalp, the face and neck, the back, the thighs and the limbs in general. Sometimes they are situated on the same side of the body as most of the bone lesions, but often they are more dispersed and not necessarily regional in distribution. A number of observers have made biopsies of such areas of pigmentation and agree that the latter reflects an increased melanin content in the basal layer of the epidermis.

In connection with the question of pigmentation, it seems worth while to call attention to the well recognized tendency of skeletal and dermal anomalies to be positively associated in other diseases also. It is demonstrated notably in relation to Recklinghausen's neurofibromatosis. It is also well known, to cite another example, that patients suffering from skeletal enchondromatosis not infrequently present congenital hemangiomas of the skin.

Premature Sexual Maturation.—For some as yet unexplained reason, pubertas praecox seems to appear only in the female patients. It is

tempting to surmise that it may represent a sex-linked defect or aberration in the development of the endocrine glands, but the problem is really not at all understood. Pubertas praecox was present in 20 of the 51 female subjects. That is, it was found in 39 per cent of these subjects, and in 22 per cent of the entire 90 cases. This incidence is certainly much higher than it would be if the cases in which premature sexual maturation is a feature were not overrepresented in the literature, on account of their special interest for pediatricians and endocrinologists.

The clinical evidences of the premature sexual maturation are: catamenia at an abnormally early age; enlargement of the external genitals, and the appearance of secondary sex characteristics, notably the development of large breasts showing prominent areolae and the growth of pubic and axillary hair. The onset of precocious menstruation often coincides more or less with the genital hypertrophy and the premature appearance of secondary sex characteristics. On the other hand, it is possible for the latter features to either precede or follow the catamenia by an interval of several months or even a few years.

The ages reported for the onset of the premature catamenia have ranged between 3 or 4 months and 10 years. The menstruation may be quite irregular as to frequency and duration. It may be accompanied, moreover, by dysmenorrhea and, at times, alarming menorrhagia. These menstrual disorders may persist until the customary age of puberty, when a normal cycle tends to be established. It appears, however, from the case described by Shallard,[16] for example, that menorrhagia in these cases can be followed after a time by complete amenorrhea. On the other hand, it seems, from one or two other reports, that such female subjects can also be fertile and go through a normal pregnancy.

The sexual precocity can hardly reflect the presence of either a granulosa cell carcinoma of an ovary or an adrenal cortical tumor. The explanation is not nearly as simple as that. Both these neoplasms, to be sure, may be the cause of precocious puberty or virilism in girls, but they tend to pursue a progressive or even malignant course, whereas the subjects in question, if no complications appear, generally survive into adult life, as long case histories show. Moreover, there is the record [20] of a young girl showing extensive skeletal changes, pubertas praecox and other manifestations of the disease who was actually subjected to laparotomy on the premise that an adrenal neoplasm might be present. However, both the adrenals and the ovaries appeared normal on inspection. Incidentally, roentgen radiation applied to the pituitary region of this patient failed to halt the progress of the disease.

On the other hand, one is impressed, on reading the pertinent case reports, by the frequency with which flat plates of the skull in such cases have shown evidence of severe involvement and, in particular, of marked

20. Freedman, H. J.: Am. J. Dis. Child. **44**:1285, 1932.

expansion and densification of the bones comprising the base of the skull. Indeed, these changes were sometimes striking enough to have led to the erroneous diagnosis of juvenile Paget's disease. It may be that this observation affords an explanation for the pubertas praecox. An affected sphenoid bone, for instance, expanded to many times its normal thickness so as to present a marked protruberance of its inner table could quite conceivably cause considerable pressure on the region of the third ventricle and of the hypothalamus in particular. Now, it is known that hypothalamic lesions, whether intrinsic or resulting from compression, may be responsible for the manifestations of precocious puberty, not uncommonly in males and occasionally also in females. However, this hypothesis is offered only as a possible explanation and not as a conviction in regard to the manifestations of pubertas praecox in the present cases. Its validity can be established or disproved only by actual autopsy observations. As a matter of fact, we incline toward the view that sexual precocity (like the rest of the endocrine aberrations and the other abnormalities in these cases) is an expression of some deeply rooted congenital defect of development, underlying the disease as a whole.

Hyperthyroidism.—In a few children suffering from fibrous dysplasia of bone, hyperthyroidism appeared after the skeletal changes were already pronounced. It is interesting to note that hyperthyroidism, unlike the sexual precocity, was not restricted to females. It may be mild and evidenced only by a moderately increased basal metabolic rate and a significantly heightened systolic blood pressure, as in the 2 cases described by Summerfeldt and Brown.[19] In the second of these cases, the basal metabolic rate was estimated at 120 per cent by the method of Benedict and Talbot. The systolic blood pressure was found elevated to 140 mm., but there were no other frank clinical evidences of hyperthyroidism. On the other hand, the child described by McCune and Bruch [2] did exhibit unmistakable clinical evidences of hyperthyroidism, in addition to showing a basal metabolic rate of + 65 per cent. Likewise, in the unusual case of Musser and Barnwell (cited in a footnote by Albright and associates [3b]), these evidences of hyperthyroidism were so pronounced that partial thyroidectomy was undertaken. The patient in this case was a boy of 11 who from the age of 9 presented an enlarged thyroid and clinical symptoms of hyperthyroidism. Specifically, his pulse rate was 128 and his systolic blood pressure was 154. He died shortly after the thyroidectomy.

Premature Skeletal Growth and Maturation.—In girls suffering from the florid form of fibrous dysplasia of bone, premature skeletal growth and maturation have occasionally been observed. Specifically, it has been noted that various centers of ossification may appear at a precocious

age and that, in general, growth may be inordinately rapid for a time, so that the affected children appear conspicuously tall and heavy for their age. For example, in the second case reported by Summerfeldt and Brown, already mentioned, the child was observed to be 49 inches (152.5 cm.) in height, compared with a normal estimated height for the sex and age of only 43 inches (109 cm.). On the other hand, there is evidence to indicate that subsequently growth in these subjects may cease prematurely, so that their adult stature may even be conspicuously stunted. Thus, in the case reported by Shallard—that of an 18 year old girl with very severe skeletal involvement, conspicuous pigmentation of skin and somewhat premature sexual maturation—growth was likewise noted for a time to be prematurely rapid as compared with that of children in her own age group. However, it apparently stopped at the age of 12, so that although the patient was notably large and heavy as a child, her mature height was no more than 4 feet 7 inches (170 cm.). The explanation for such precocious skeletal growth and maturation is admittedly a matter for conjecture. In the first case cited, there was also clinical evidence of hyperthyroidism; in the second case, such evidence was lacking.

Cardiovascular and Other Developmental Anomalies.—With respect to developmental anomalies, some of the autopsy observations in Coleman's [11] case are revealing. Specifically, in that case, coarctation of the aorta and a small rudimentary left kidney were found. Stauffer, Arbuckle, and Aegerter [81] have observed the case of a young man of 19 who presented, in addition to extensive skeletal changes and a large brownishly pigmented patch on his back, multiple congenital arteriovenous aneurysms in the left upper extremity and apparently a similar condition in the left lower extremity.

These findings lend additional support to the opinion we have held for some time, that fibrous dysplasia in its severe form is an expression of a deeply rooted defect of development, in which the basic clinical picture, represented by the skeletal changes, is amplified by the various extraskeletal abnormalities.

PROGNOSIS AND TREATMENT

No hard and fast rule can be laid down for treatment of the lesions in bone, since in any particular case what is or is not done should be guided mainly by clinical indications. That is, procedure should be governed not by the size and the number of the lesions but by their troublesomeness or their failure to cause trouble. Thus a solitary lesion uncovered only incidentally in the course of a routine physical examination can in many instances safely be left entirely alone after the diagnosis

has once been established, perhaps with the aid of a biopsy. In a case of polyostotic involvement, it is again only such lesions as are causing difficulty that really require attention.

In a case in which only one bone is affected and the lesion in it is small, thorough curettage of the latter and filling of the cavity with autogenous bone chips may reasonably be expected to eradicate the disease. On the other hand, when the solitary lesion is in a rib, for instance, it may be wiser to resect than to curet it. If it is still only one bone that is involved but the lesion is large and the area affected is one subjected to heavy loading (notably the upper end of a femoral shaft), the insertion of a massive autogenous bone graft is advisable. In fact, such a graft may have to be used subsequently in a case in which curettement and autogenous bone chips were used at first but in which the chips were resorbed and the lesion recurred. Indeed, the principle of giving mechanical support through the introduction of a massive graft is usually appropriate for an affected bone which is in danger of fracturing or has already undergone one or more fractures. In the gravest cases it is very likely that this may have to be done at least to an affected femur to overcome pain and prevent the development of a crippling deformity. Especially in such cases it will usually also be necessary to use such nonoperative aids as corrective shoes, braces and corsets for overcoming skeletal shortening, weakness and deformities.

Aside from these purely mechanical (surgical and nonsurgical) measures, an individual case may or may not require additional attention. Patients showing involvement of one bone usually do not need this, beyond the caution to be careful in the use of the affected part, even after surgical intervention if the latter has not completely eradicated the lesion. Patients showing involvement of several bones of a limb with or without an adjacent part of the trunk and patients showing severe skeletal involvement require the same caution, for in these cases whatever surgical work is done is directed merely toward the correction of deformities and fractures in strategic areas. In any case, nothing can be done for the abnormal pigmentation of skin, and probably nothing should be done for the pubertas praecox or even the hyperthyroidism occasionally present in the cases of severe involvement.

The outlook as a whole need not be too pessimistic even in the cases of severest disease. It is true that the life expectancy is short in some of these, particularly the ones in which the associated extraskeletal (and especially glandular) abnormalities are already present in early childhood. However, the patients presenting only limited or moderate expressions of the disorder seem to have a normal life expectancy, and those in whom only one bone is affected certainly do. Furthermore, so far as the skeletal manifestations of the disease are concerned, lesions which were

present before the end of the period of active growth often seem to slow down spontaneously in their activity and even become arrested. Finally, it is important to point out that we do not know of a single instance, regardless of the extent of the skeletal involvement, in which the lesion or lesions of bone have undergone malignant transformation.

SUMMARY

In any individual case of the disorder which we are calling fibrous dysplasia of bone, one, several or many bones may be implicated, and, especially in the cases in which the skeletal involvement is severe and has appeared early in life, certain extraskeletal abnormalities may also be present as part of the total disease picture.

As to the skeletal aspect of the condition, emphasis was laid on the fact that when more than one bone is affected, the bones involved are likely to be solely or mainly on one side of the body. In an affected bone, the area implicated may be found expanded in part or throughout. Where it is not expanded, the regional cortex is likely to show at least erosion and thinning from the medullary side. The interior of the involved area is found to be filled mainly by an evenly whitish or reddishly speckled rubbery and compressible tissue. Fundamentally, this is fibrous connective tissue. It may be gritty throughout from the presence everywhere in it of newly formed trabeculae of immature bone. Or, instead, it may show some smaller or larger, nongritty, highly collagenous areas in which few if any bony trabeculae are to be seen. In some lesions, islands of hyaline cartilage may also be present within the fibrous connective tissue. Furthermore, in an occasional lesion, focal degeneration of this tissue may have led to the formation of small secondary cysts. Altogether, as we have tried to show, the gross and microscopic skeletal features are quite adequate for clear delimitation of the condition.

As to the extraskeletal aspects of the condition, it was pointed out that abnormal pigmentation of skin and (in females) premature sexual development are the two most common. Reference was also made to the presence, in a few instances, of hyperthyroidism, ephemeral premature skeletal growth and maturation, and even a history of very grave icterus in early infancy. In 1 case cited, multiple congenital arteriovenous aneurysms were present in the left upper extremity and apparently a similar condition in the left lower extremity, while in another case the aorta showed coarctation and the left kidney was small and rudimentary.

When one looks at the disease as a whole (skeletal and extraskeletal), one sees much to support the idea that it has its basis in a defect of development in which the central clinical picture, represented by the skeletal lesions, is amplified by the various extraskeletal abnormalities.

As to the skeletal lesions in particular, these apparently result from perverted activity of the specific bone-forming mesenchyme, and we have attempted to suggest this derivation by the name "fibrous dysplasia of bone." Indeed, this name is also appropriate for the disease as a whole, since, as noted, the skeletal lesions constitute the central feature of the clinical syndrome and it is mainly in some of the graver cases that non-skeletal abnormalities are present in addition.

The present paper also devotes attention to the clinical (including roentgenographic) aspects of fibrous dysplasia of bone. The need for differentiation of severe fibrous dysplasia of bone from hyperparathyroidism with severe skeletal involvement is also brought out. In addition, some broad rules for the management of fibrous dysplasia have been set down. Finally, it was pointed out that we know of no cases of fibrous dysplasia of bone in which any of the lesions have undergone malignant transformation, irrespective of the extent of the skeletal involvement.

COMPARATIVE PATHOLOGY OF EPIDEMICS OF POLIOMYELITIS OCCURRING IN LOS ANGELES COUNTY IN 1934-1935 AND 1939

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LOS ANGELES

A preliminary report on the 1934 epidemic of poliomyelitis in Los Angeles County from the standpoint of pathology was made by us [1] at the annual meeting of the American Public Health Association held at Pasadena, Sept. 3, 1934. Owing to circumstances largely beyond our control the more complete study was not finished until recently. Since about a dozen persons who died of poliomyelitis came to autopsy at the Los Angeles County Hospital during an epidemic in 1939, it was decided to make a comparative study of the pathologic aspects of the two epidemics.

There were so many unusual clinical aspects to the 1934 epidemic that a number of workers refused to believe that the epidemic was caused by the virus of poliomyelitis. Some of these features may be reviewed briefly. A considerable number of cases were diagnosed as early as May, which is earlier than most outbreaks occur. In 1934, according to Dunshee and Stevens,[2] only 44 per cent of the patients were children under 10 years, while in the epidemic in California in 1912 78 per cent of the patients were children under 8 years of age. The total incidence for Los Angeles County (including the city), according to Stevens,[3] was 1,792, with 25 deaths—a case mortality rate of 1.39 per cent. The total number of cases in the state of California was 2,732 (Sept. 6, 1934). In Fresno County, which ranked second to Los Angeles County in number of cases, the mortality rate was 2.3 per cent. These rates are remarkably

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1. Van Wart, R. M.; Courville, C. B., and Hall, E. M.: Am. J. Pub. Health **24**:1207, 1934.

2. Dunshee, J. D., and Stevens, I. M.: Am. J. Pub. Health **24**:1197, 1934.

3. Stevens, G. M.: Am. J. Pub. Health **24**:1213, 1934.

low, according to Leake and associates.[4] The unusual clinical manifestations of the 1934 epidemic have been discussed by Bower and co-workers.[5] These authors stated that the symptoms were milder and less crippling than usual. Early neurologic localized signs which help to differentiate preparalytic poliomyelitis from the systemic phase of other acute infections were often absent. Diarrhea with severe abdominal cramps, vomiting and other gastrointestinal symptoms were common. The degree and the duration of muscle pain and muscle tenderness were out of proportion to the motor phenomena. The treachery of the disease was more marked than usual. On the other hand, rapid and apparently complete recovery occurred in some patients who early appeared to be doomed to extensive residual paralysis. Sensory phenomena were prominent and varied. Hypesthesia paresthesia and hyperesthesia, severe pain and sweating were frequent. These signs were often present in the extremity opposite the one in which motor paralysis was present. Lesions in the cord were at times severe on one side and mild on the opposite side, explaining, no doubt, these physical findings.

Bigler and Nielsen [6] in their report on the neurologic characteristics of the disease in adults in 1934 cited several interesting cases. Their first case was that of a woman of 35 years who had an acute onset of "neuritic" pain, marked weakness, tenderness which was generalized but more severe over the nerve trunks, fever, transient paralysis, nonpitting leathery edema, sweating, extreme hypersensitivity, emotional instability and muscular spasms. There was marked muscular atrophy of the hands, and arthritic changes were present. The acute symptoms became intensified at the end of the tenth week and again after seven and one-half months. After eight months the patient was still confined to bed, unable to walk. Motion was limited more by pain than by muscular weakness. Exacerbations lasting a week were still recurring after a period of two years. The authors considered these symptoms quite typical of the adult group. Their second case was that of a single woman of 49 years in whom psychic blindness developed—her mind failed to interpret the things she saw. In addition, she had many of the symptoms just enumerated. Two of the patients observed by Bigler and Nielsen had tabes complicating the acute symptoms of poliomyelitis. One patient (case 4) died and at autopsy presented marked softening of the cord.

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4. Leake, J. P.; Cedar, E. T.; Dearing, W. P.; Gilliam, A. G., and Chope, H. D.: Am. J. Pub. Health **24**:1204, 1934.

5. Bower, A. G.; Meals, R. W.; Bigler, M. F.; Ewing, J., and Houser, V.: Am. J. Pub. Health **24**:1210, 1934.

6. Bigler, M. F., and Nielsen, J. M.: Bull. Los Angeles Neurol. Soc. **2**:47, 1937.

The more important laboratory and clinical data obtained in our study are shown in table 1. It will be noted that the number of cells in the spinal fluid was relatively low. This correlates well with the low

TABLE 1.—*Clinical and Laboratory Data—1934-1935*

Sex of Patient; Age	Duration, Days	Temperature, F.	Spinal Fluid				Blood		Paralysis
			Pressure, Mm.	Cells	Kind of Cells	Pandy Reaction	White Cells	Differential Count	
M 5	4	103	150	77	81% polymorphonuclears, 23% lymphocytes	+	.....	....	Arm, slight
F 15	5	100.6	...	..	.....	..	.....	....	Respiratory system
M 28	5½	97.8	175	10	2% lymphocytes	Trace	.....	....	Diaphragm
M 24	6	102	...	28	23% lymphocytes, 5% polymorphonuclears	+	.....	....	Legs, arms and respiratory system
M 13	5½	103.2	275	50	30% red cells, 20% polymorphonuclears and lymphocytes	Trace	.....	....	Respiratory and laryngeal muscles
F 37	30	97.6	120	41	41% lymphocytes	0	.....	....	Ocular muscles and bladder
F 18	4	99.6	220	227	66% polymorphonuclears, 34% lymphocytes	+	.....	....	Thoracic and respiratory muscles
F 27	15	100.8	...	..	.....	..	.....	....	Respiratory and pharyngeal muscles
M 12	7	105	225	24	20% lymphocytes, 4% polymorphonuclears	Trace	11,200	85% polymorphonuclears	Thoracic muscles
M 12	8	101	275	49	46% lymphocytes, 3% polymorphonuclears	++	15,500	83% polymorphonuclears	11th nerve
F 15	9	102.8	190	40	All lymphocytes	+	12,000	82% polymorphonuclears	
F 7	5	103.6	250	48	44% lymphocytes	+	.....	....	Both arms, partial
F 11	6	.....	...	..	.....	..	.....	....	Respiratory system
M 20	4 mo.	98	125	14	All lymphocytes	+	15,900	73% polymorphonuclears	
M 20	4	101.6	...	100	.....	Normal	.....	....	Respiratory system
F 8	4	102	300	..	Numerous lymphocytes	0	.....	....	Laryngeal muscles
M 18	11	102.8	220	280	Many lymphocytes	?	.....	....	
M 12	30	100	200	165	All lymphocytes	0	18,000	....	Pharyngeal muscles and respiratory system

incidence of paralysis. The great majority of inflammatory cells in the spinal fluid were lymphocytes. In 2 cases in which the disease was of only four days' duration the greater percentage of the cells were poly-

morphonuclear leukocytes. Lesions in the brain stem and the cord were pronounced in the case of a girl aged 18 years. Although the temperature on admission ranged from subnormal to 105 F., it was usually between 101 and 102 F. Only five complete blood counts were done in the 1934-1935 epidemic. In these the white blood cells ranged from 11,200 to 18,000 and the polymorphonuclear leukocytes ranged from 73 to 85 per cent. In only 3 cases did paralysis of the extremities develop, and in 2 of these it was said to be "slight" and "partial," respectively.

An outstanding feature of this epidemic was the exceptionally high degree of communicability. Especially affected were workers in the Los Angeles County Hospital. Stevens [3] reports that 137 employees contracted the disease, including 18 staff doctors and interns. Leake and co-workers [4] stated that multiple cases were reported from 12.5 per cent of the households where cases occurred. This figure is probably high, as Aycock [7] stated that poliomyelitis shows little tendency to spread within the household.

Leake and associates, in their discussion of the epidemiology of the 1934 epidemic in California, appeared not to have doubted the presence of the virus of poliomyelitis. In their concluding paragraph, they stated: "All the evidence at hand indicates that the mode of spread in this epidemic is similar to that which is usually accepted for poliomyelitis, namely, by contact with human carriers, and to a much less extent, with recognized cases of the disease."

At autopsy, the central nervous system was exposed aseptically, and from various parts of the brain and cord material was taken for virus studies. A suspension of the material was inoculated intracerebrally into monkeys (Macacus Rhesus) by Kessel and associates.[8] In many of the animals inoculated paralysis of the extremities typical of experimental poliomyelitis developed. Inoculations of the virus material were made in 16 of 19 cases. Symptoms of poliomyelitis developed in 11 of the monkeys, while 5 animals failed to react. Eighty-four per cent of the monkeys recovered without residual paralysis. Those that died during the course of the disease had lesions in the medulla and the spinal cord which were characteristic of poliomyelitis.

Poliomyelitis virus was isolated from the nasal washings of 1 patient by Paul, Trask and Webster.[9] Six monkeys inoculated intracerebrally with this virus all presented characteristic symptoms with paralysis.

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7. Aycock, W. L.: The Epidemiology of Poliomyelitis, in Virus and Rickettsial Diseases with Especial Consideration of Their Public Health Significance: Symposium, Cambridge, Mass., Harvard University Press, 1940, p. 555.

8. Kessel, J. F.; Van Wart, R.; Fisk, R. T., and Stimpert, F. D.: Proc. Soc. Exper. Biol. & Med. **35**:326, 1936.

9. Paul, J. R.; Trask, J. D., and Webster, L. T.: J. Exper. Med. **62**:245, 1935.

METHODS

For our purpose the materials from the two epidemics were prepared in a similar way. The brain and cord were fixed in 4 per cent solution of formaldehyde. Each brain was fixed separately, at least a gallon of solution being used. The latter was renewed several times during the first two weeks. After the brains were thoroughly hardened, blocks were cut from the cerebral cortex, the hippocampus, the basal nuclei, the cerebellum, the pons and the medulla. Blocks were taken also from two levels of the cord, preferably the cervical and the lumbar. All blocks were embedded in paraffin, cut at 8 to 10 microns and stained with hematoxylin-eosin and gallocyanin. We found the latter to be an excellent stain for cellular detail, especially valuable in the estimation of degenerative changes in the large motor cells of the anterior horns.

ORGANIC CHANGES OTHER THAN THOSE OF THE CENTRAL NERVOUS SYSTEM IN BOTH EPIDEMICS

It is generally considered that poliomyelitis manifests itself not only by acute inflammatory changes in the central nervous system but also by evidences of acute inflammation in the systemic organs. In both the 1934 and the 1939 epidemic there was observed acute hyperemia of many organs. The lymphatic system was noticeably affected, as is usually the case. The mesenteric lymph nodes were as a rule swollen and hyperemic. The spleen and the thymus were also enlarged in numerous instances. In the 1934 epidemic the mesenteric lymph nodes were enlarged in two thirds of the cases; no record was made concerning these structures in 5 cases. The spleen was enlarged in 50 per cent of the cases, while in 7 cases it was somewhat smaller than the average for that age. The enlargement of the spleen was more frequently observed in adults. The condition of the thymus was usually not recorded in this group.

In the 1939 epidemics the mesenteric lymph nodes were enlarged in 80 per cent of the cases. The thymus was often enlarged, while the spleen was enlarged in 50 per cent of the cases, as it was in the 1934 group.

Hyperemia was usually observed in the liver, the kidneys and the adrenals as well. The sympathetic ganglions along the lumbar portion of the spine were as a rule distinctly reddened. Petechial hemorrhages occurred in the epicardium in a number of instances. Microscopically, the organs showed acute congestion and some edema. The glandular organs exhibited more or less parenchymatous degeneration.

GROSS CHANGES IN THE CENTRAL NERVOUS SYSTEM IN BOTH EPIDEMICS

There was no essential difference in the gross appearance of the brain and spinal cord in the two epidemics. Without exception, the leptomeninges of the brain were diffusely reddened, sometimes intensely.

The cut surfaces of the brain when examined in the fresh state were dusky pink, with red injected vessels showing here and there. The leptomeninges of the cord were often intensely congested. When it was found necessary to transect the cord in the fresh state, for purposes of the virus study, the white and to a lesser degree the gray substances were soft and edematous and bulged markedly from the cut surfaces. In several instances the cord was so soft it was distinctly mushy. Presumably, this condition was due to circulatory failure resulting from the extreme stasis.

MICROSCOPIC CHANGES IN THE CENTRAL NERVOUS SYSTEM IN THE 1934-1935 EPIDEMIC

The changes in the cerebral cortex and the hippocampus were confined to hyperemia, edema and occasional small hemorrhages. These changes were even more prominent at the cervical and lumbar levels of the cord (fig. 1 *A*). There was slight cuffing in the cerebral cortex in 3 cases, and slight cellular infiltration in 3 cases. No substantial degeneration of the neurons was observed. A moderate increase in the number of oligodendroglia cells was noted. Satellitosis was fairly prominent in a number of instances. In the cerebellum and the basal nuclei in 5 or 6 cases there was slight cuffing of some of the vessels with small round cells, as well as slight or questionable cell degeneration. Satellitosis was noted frequently in this group. In 5 of the 18 cases with autopsy in 1934-1935, no sections of the pons were available, all of this material having been taken for virus studies. Of the 11 cases studied, 9 showed cuffing or cellular infiltration and some degree of cellular degeneration. In 3 cases the changes were recorded as moderate (++). In 2 cases the medulla was not seen microscopically because of a shortage of material.

Cellular Infiltration and Cuffing.—In passing from above downward, cellular infiltration or lymphocytic cuffing about the vessels of the nervous system was first encountered to any degree in the medulla. In only 1 case was the perivascular cuffing severe (fig. 1 *B*). In the cord, however, this type of infiltration was severe in 5 cases. In 3 of 18 cases no cellular infiltration of the medulla or the cord was observed, while in 2 others the amount was slight. In the cord, diffuse infiltration was pretty well confined to the gray matter, while perivascular cuffing was seen frequently in the white substance as well. In some of the cases in which the more severe early stage was present, many polymorphonuclear leukocytes were present. The great majority of the infiltrating cells consisted of microglia and the macrophages derived from these cells. The macrophages as well as the neutrophils surrounded

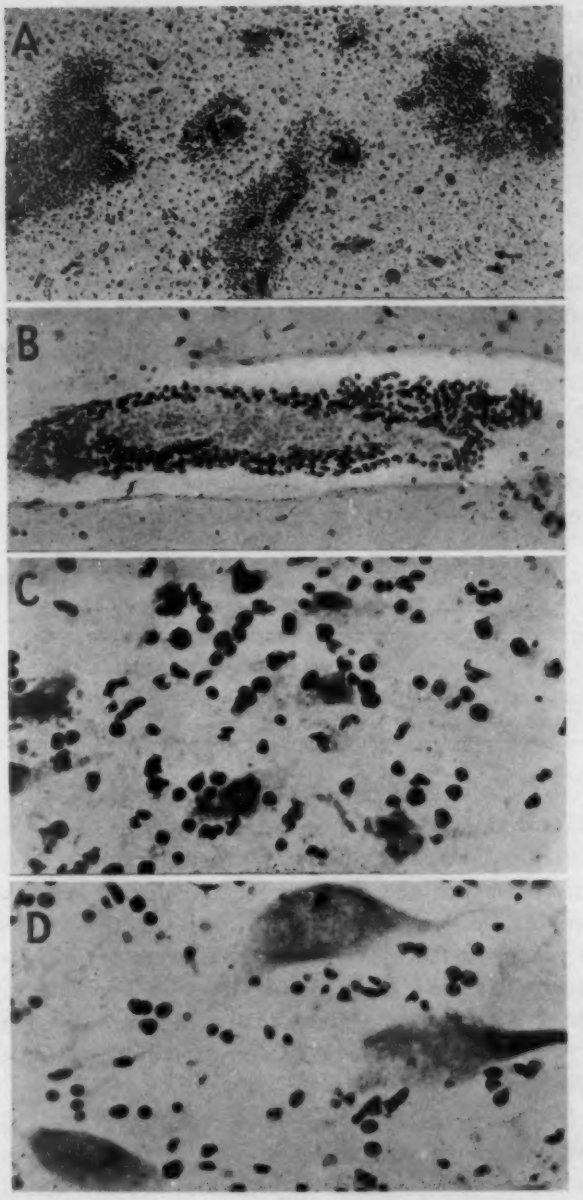

Fig. 1.—*A*, hemorrhages into the cervical portion of the cord (1934). Note injection of the small vessels and edema of the gray substance. Hematoxylin and eosin; × 100. *B*, lymphatic infiltration about a small blood vessel in the medulla (1939). This picture is typical of both epidemics. Gallocyanin; × 240. *C*, phagocytosis of dead neurons by macrophages (neuronophagia) (1934). Gallocyanin; × 360. *D*, chromatolysis of motor cells in anterior horn of lumbar cord (1934). Gallocyanin; × 450.

dead motor neurons and engulfed them. In several instances neuronophagic activity was marked (fig. 1 *C*). In a general way the intensity of the cellular infiltration was a measure of the degree of motor cell destruction. The most intense reactions were seen in the anterior horns of the gray substance. Considerable infiltration, especially of the perivascular type, was likewise seen in the posterior horns of the cord.

Nerve Cell Degeneration.—The most significant changes in poliomyelitis are the nerve cell degeneration and necrosis in the brain stem and in the spinal cord. Considering the high incidence of infection and the low incidence of residual paralysis in the 1934 epidemic, one would expect only mild or early motor cell changes. Yet in the cases in which death occurred and the body came to autopsy there was a considerable amount of cell destruction among the motor neurons of the anterior horns (table 2).

It is evident from table 2 that the changes were slight or moderate in the majority of cases. This correlates well with the clinical evidence

TABLE 2.—*Degree of Change in the Motor Nerve Cells in Epidemic of 1934 (19 Cases)*

	Slight	Moderate	Marked	No Sections
Cervical cord	4	12	3	0
Lumbar cord	7	4	6	2

that paralysis of the extremities was not prominent. The survival period was only one week or less in 11 of the 19 cases. Apparently the virus reached the spinal cord very promptly, for there seems to be no correlation between the duration of the disease and the severity of the lesions in the lumbar part of the cord. In the 6 cases in which the lesions of the lumbar part of the cord were severe, the duration of the disease was as follows: in 1 four days, in 3 five days, in 1 eight days and in 1 fifteen days. Thus, of 6 patients, 4 lived less than a week after the onset of illness. The great majority of patients died of bulbar paralysis, according to the records.

Cytologic Changes in Degeneration of Anterior Horn Cells.—It has already been stated that the changes in the structure of the cell are usually accompanied by cellular infiltration of the interstitial gray substance. In the normal cell stained with toluidine blue or gallocyanin the Nissl substance stands out conspicuously. It is regimented into more or less concentric rows about the centrally placed nucleus. The nucleus is clear, with a large slightly eccentric nucleolus. The first transformations within the cell consist of "acute swelling" with fusing and

TABLE 3.—*Quantitative Estimate of Pathologic Changes in Each Patient Recorded for Four Different Levels of the Nervous System—1934-1935 Epidemic*

Sex of patient	M	F	M	M	M	F	F	F	M	M	F	F	F	M	M	F	M	M	F
Age of patient, years	5	15	28	24	13	37	18	27	12	12	15	7	11	20	20	8	19	12	8
Survival period	4 days	5 days	6 days	5 days	6 days	1 mo.	4 days	15 days	7 days	8 days	9 days	5 days	4 days	4 mo.	4 days	4 days	11 days	1 mo.	21 mo.
Congestion																			
Cerebrum	+++*	++	++	++	++	++	++	+	++	+	+	++	+	++	+++	++	++	+++	++
Medulla	+++	+++	++	+++	++	++	++	++	++	++	+	++	+++	+	+++	++	+++	+++	++
Cervical cord	++	+++	+++	+++	+++	++	+++	+++	++	++	++	+++	+	++	+++	++	+++	++	+
Lumbar cord	+++	+++	+++	+++	++	++	+++	++	++	++	++	++	++	+	..	++	..	++	+
Hemorrhage																			
Cerebrum	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	0	0	+	+
Medulla	0	+	+	0	+	+	+	0	+	0	0	+	++	0	+	+	+	++	++
Cervical cord	++	+++	+	+++	+	+	++	+	+++	+	+	+++	0	0	..	0	0	+	0
Lumbar cord	0	+	+	+	0	+	+	++	+	0	+	+	0	0	..	0	..	+	0
Cellular infiltration																			
Cerebrum	+	0	0	0	0	0	0	+	0	0	0	+	0	0	0	0	0	0	0
Medulla	+++	+	0	0	++	+	++	+	0	+	+	++	+	+	++	++	++	+	+
Cervical cord	++	+++	++	+++	++	+	+++	++	+	+	+	+++	++	+	++	+	++	+	+
Lumbar cord	++	+++	+	+++	0	++	+++	++	+	+++	+	+++	++	+	..	+	..	+	+
Lymphoid Cuffing																			
Cerebrum	+	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	+	0	0
Medulla	++	+	0	+	++	+	+++	++	+	+	+	+	++	0	++	++	++	+	+
Cervical cord	++	+++	++	+	+	+	+++	+++	0	+	+	+++	++	0	++	+	++	+	+
Lumbar cord	++	++	+	+	+	+	+++	+++	0	++	++	+++	+++	0	..	+	..	+	0
Nerve cell degeneration																			
Cerebrum	0	0	0	[illegible]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Medulla	+++	+	..	..	+	+	+++	++	+	+	+	++	+	+	++	+++	++	++	+
Cervical cord	++	+++	++	+++	++	++	++	+++	++	+	+	++	++	+	++	++	++	++	+
Lumbar cord	+	+++	+	+++	+	++	+++	+++	+	+++	++	+++	++	0	..	++	..	+	+

* Explanation of signs: 0, no changes; +, slight changes; ++, moderate changes; +++, severe changes.

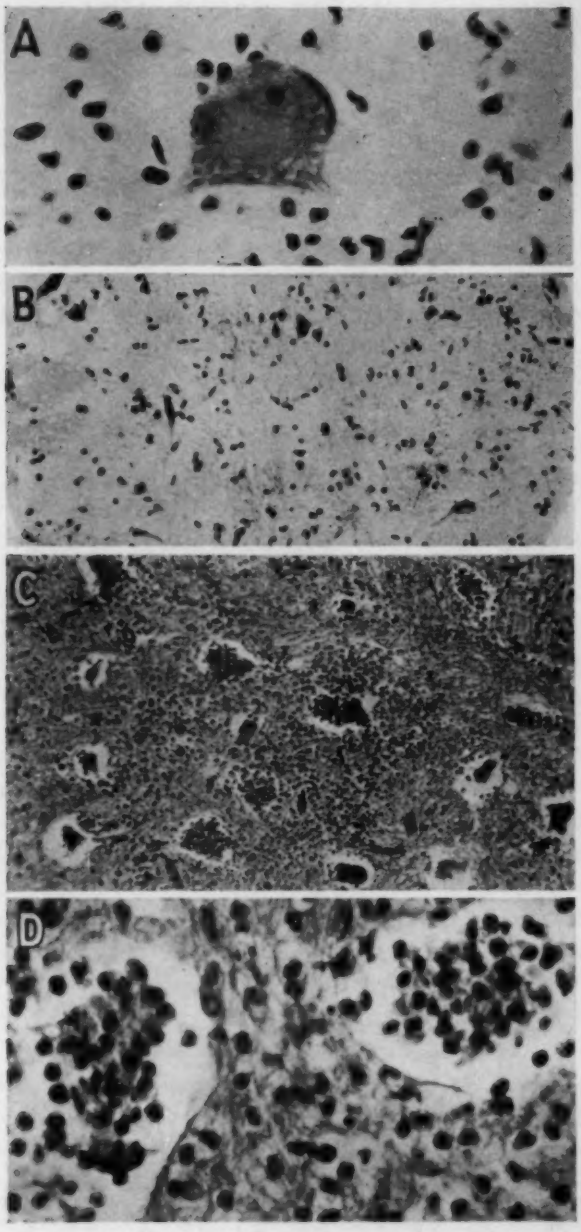

Figure 2

(See legend on opposite page)

smudging of the tigroid material (chromatolysis). With fusion of the Nissl substance the nucleus becomes obscured, owing to the smudgy staining of the cell (fig. 1 *D*). A later stage shows a fine network or at times a single row of Nissl particles about the periphery of the cell, with finely granular material occupying the central area. There is often a zone of perinuclear pallor. The nucleus is usually near one pole of the cell and is often rather inconspicuous because of partial lysis. Small vacuoles, probably the result of hydropic degeneration, frequently are seen about the periphery within the network of Nissl particles (fig. 2 *A*). More advanced changes leave the cells in various stages of acute disintegration, surrounded by engulfing macrophages. One patient, a girl of 8 years, survived twenty-one months after the onset of the disease. She died after an apparent exacerbation of poliomyelitis. Sections of the lumbar portion of the cord show moderate cellular infiltration of the gray substance. Only a few sclerotic, darkly staining motor cells remain (fig. 2 *B*). The picture is quite different from that seen in the cases of acute involvement.

A quantitative estimation of the changes found at four different levels in the nervous system (the cerebrum, the medulla and the cervical and the lumbar portion of the cord) are recorded for each patient in table 3.

CLINICAL COMPARISON OF THE EPIDEMIC OF 1939 WITH THAT OF 1934

From the clinical standpoint the 1939 epidemic was mainly of the usual order. The unusual and bizarre features that had harried the medical attendants in 1934 were largely absent; however, a small number of cases were more or less atypical.

In the state of California 1,002 cases were reported, while in Los Angeles County (including the city) there were 361 cases, with 24 deaths. The mortality rate in the county, therefore, was 6.64 per cent. In

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EXPLANATION OF FIGURE 2

A, further stage in neuron degeneration—nucleus pale, eccentrically placed in the cell; central Nissl substance gone, leaving a peripheral zone of fine Nissl material with small vacuoles within the meshes (1934). Gallocyanin; × 500. *B*, atrophic, dark-staining "sclerotic" nerve cells in one of the anterior horns in a case of poliomyelitis that terminated after twenty-one months (1934). Note the sparsity of neurons and the moderate diffuse infiltration of microglial cells. Gallocyanin; × 150. *C*, marked necrosis of nerve cells with extensive phagocytosis and macrophage activity in an anterior horn of the lumbar portion of the cord (1939). Many of the infiltrating cells are polymorphonuclear leukocytes. Gallocyanin; × 100. *D*, higher power of *C*. Many of the phagocytes are polymorphonuclears. Hematoxylin and eosin; × 450.

1934 there were 2,732 cases in the state and 1,792 cases in the city and county of Los Angeles (Stevens [3]). The mortality rate was 1.39 per cent. Thus it is seen that the incidence of poliomyelitis in 1939 was less than in 1934, while the mortality rate was much higher. The 1939 epidemic is, therefore, more comparable to the general run of epidemics

TABLE 4.—*Clinical and Laboratory Data—1939*

Sex of Patient; Age	Duration, Days	Temperature, F.	Spinal Fluid				Blood		Paralysis
			Pressure, Mm.	Cell Count	Kind of Cells	Globulin	White Blood Cells	Differential Count	
M 14	11	101.4	225	1,052	All lymphocytes	+	10,300	80% polymorphonuclears	Left thigh and leg
M 24	5	101.6	...	325	90% lymphocytes	Slight trace	.....	....	Diaphragm
M 13	4	103	250	130	60% lymphocytes	+	.....	40% polymorphonuclears	0
M 21	11	100.4	260	102	90% lymphocytes	+	.....	....	0
F 12	..	100	170	146	Red blood cells and lymphocytes	..	.....	....	0
M 10	..	105	225	1,171	All lymphocytes	..	.....	....	0
M 15	4	102.2	275	160	Mostly lymphocytes	+	.....	....	Arms and bladder
F 7	9	.....	350	380	Mostly lymphocytes	+	.....	....	0
M 31	8	103.6	225	325	All lymphocytes	Slight trace	12,100	78% polymorphonuclears	Pharynx
M 14	5	101	225	276	82% lymphocytes	+	19,300	76% polymorphonuclears	0
F 20	12	102	125	200	Predominantly lymphocytes	Trace	10,000	73% polymorphonuclears	Pharynx (slight), weakness of leg

of poliomyelitis, such as was seen in California in the years 1912, 1927 and 1930.[2]

The more important laboratory and clinical data for the 1939 epidemic are presented in table 4. The cell counts of the spinal fluid were higher than in the earlier epidemic. Counts of 1,052 and 1,171 were recorded respectively in 2 cases. Neither one of the patients, however, showed severe lesions of the brain stem and cord. Lymphocytes predominated in all cases. The temperature range was from 100 to 105 F. The average, however, was between 101 and 102 F. Only four blood counts

are recorded for this series. The white cells ranged from 10,200 to 19,300 cells per cubic millimeter and the polymorphonuclear leukocytes from 73 to 80 per cent, figures which are closely comparable to those for these factors in the 1934-1935 epidemic. Paralysis of one or more of the extremities is recorded in only 2 cases, while weakness of the leg is referred to in 1 case.

COMPARISON OF MICROSCOPIC OBSERVATIONS IN 1939 WITH THOSE IN 1934-1935

There was nothing unusual in the gross appearance of the pathologic material in 1939. The microscopic alterations varied in 1939 from those in the earlier epidemic in detail but not in kind. A comparison of table 5 with table 3 makes it evident that hemorrhages into the cervical part of the cord were less severe in the 1939 as compared with the earlier epidemic. Cellular infiltration and lymphoid cuffing were more extensive in the cord in the earlier epidemic. On the other hand, motor cell destruction was more uniformly severe in 1939 than it was in the years 1934 and 1935 (fig. 2 *C* and *D*). The changes were severest in the cervical rather than in the lumbar portion of the cord. In 3 instances there was severe cell destruction on one side of the cord while the process was only slight or moderate on the opposite side.

Since the sensory symptoms were so pronounced in the patients in 1934-1935, an effort was made to investigate changes that might occur in the sensory apparatus. Accordingly specimens were taken from some of the dorsal root ganglions, the celiac plexus, the sympathetic ganglions of the lumbar region and some of the peripheral nerves in a number of the cases in which bodies came to autopsy. This material was fixed in solution of formaldehyde and cut by the freezing microtome. The following stains were employed: The more cellular parts were stained with hematoxylin and eosin, scarlet red, gallocyanin, Cajal's reduced silver and Bielschowsky's silver stain. The nerve fibers were stained with hematoxylin and eosin, scarlet red, a modified Spielmeyer stain for myelin sheaths and Cajal's reduced silver stain.

This work was carried out by one of us (C. B. C.), but no positive findings of significance were obtained. In spite of the marked symptomatic sensory changes, little or no permanent damage could be demonstrated anatomically. The virus was apparently highly irritating but of low virulence, since it produced a pseudoneuritic type of disease instead of a true neuritic type with anatomic lesions in the peripheral nerves and ganglions.

TABLE 5.—*Quantitative Estimate of Pathologic Changes in Each Patient Recorded for Four Different Levels of the Nervous System—1939 Epidemic*

Sex of patient	M	M	M	M	F	M	M	F	M	M	F	M	M
Age of patient, years	14	24	13	21	12	10	15	7½	31	14	20	13	46
Survival period	10 days	6 days	3 days	7 days	9 days	4 days	2 days	8 days	6 days	3 days	12 days	3-4 weeks	
Congestion													
Cerebrum	++*	++	++	++	++	+++	++	++	++	++	+++	++	..
Medulla	++	++	++	++	++	++	+++	++	++	+++	+++	++(e)	+
Cervical cord	+++	+++	+++	+++	++	+++	+++	++(t)	++	+++	++	++	+(t)
Lumbar cord	..	+++	++	+++	++	+++	+++(e)	+	+++(e)	++	+++	+++	+
Hemorrhage													
Cerebrum	+	0	+	0	0	0	0	0	+	0	+	0	..
Medulla	0	0	0	0	+	0	+	0	0	+	0	0	0
Cervical cord	++	0	0	0	++	0	+	+(t)	+	0	0	0	0
Lumbar cord	..	0	0	0	+	+	0	0	+	0	0	0	0
Cellular infiltration													
Cerebrum	0	+	+	0	0	0	+	0	+	+	0	+	..
Medulla	+	+	+	+	+	++	++	+	+	++	+	++	+
Cervical cord	+	++	++	+	+	+	+	++(t)	+	++	+	++	+
Lumbar cord	..	+	+	+++	++	+	++	++	++	++	++	++	+
Lymphoid cuffing													
Cerebrum	0	0	0	0	0	0	+	0	+	0	0	+	..
Medulla	+	++	+	++	++	++	++	++	++	++	+	++	+
Cervical cord	+	++	+	++	+	++	+	+(t)	+	+	++	++	+
Lumbar cord	..	++	+	+	++	+	++	+	++	+	+	++	+
Nerve cell degeneration													
Cerebrum	0	0	0	0	0	0	0	0	0	0	0	0	..
Medulla	+	+	+	+	0	+	+	+	+	+++	+	+	+
Cervical cord	+++	+++	+++	+++	+++	+++	+++	+++(t)	++	++	+	+++	+
Lumbar cord	..	++	+	++	++	+	+++	+	+++	+	+++	+++	..

* Explanation of signs: 0, no changes; +, slight changes; ++, moderate changes; +++, severe changes. The letter t in parentheses signifies thoracic cord; e, edema.

It was suggested that a careful study of the white matter immediately surrounding the gray part of the cord might furnish an explanation of the unusual sensory phenomena. The results of this investigation are given in table 6. Considering the difference in the number of cases (18 in 1934-1935 and 12 in 1939), the results are as nearly the same as one might expect. In keeping with the changes found in the gray substance of the cord, congestion was somewhat more intense in the 1934-1935 group.

In table 7 again, when one considers the difference in number of cases in the two series, the results appear quite similar. If anything, the reaction is slightly more intense in the 1939 group.

TABLE 6.—*Reaction in the White Matter of the Spinal Cord*

	1934-1935		1939	
Degree of Change	Congestion	Lymphocytic Cuffing	Congestion	Lymphocytic Cuffing
Slight	10	11	3	7
Moderate	8	5	9	5
Marked	1	1	1	..

TABLE 7.—*Reaction in the Posterior Horns of the Spinal Cord*

	1934-1935			1939		
Degree of Change	Congestion	Cellular Infiltration	Cellular Degeneration	Congestion	Cellular Infiltration	Cellular Degeneration
Slight	10	12	3	3	3	1
Moderate	5	4	2	6	6	1
Marked	2	..	..	1	2	..

COMMENT

It is evident from the tables that great similarity exists in the data presented. The graphs (fig. 3) based on the pathologic observations in the two epidemics also show practically identical curves. Furthermore, these curves correspond closely with those presented by Landon and Smith,[10] based on their 81 cases of poliomyelitis. The same factors are used in making our graphs as were used by Landon and Smith, so that a direct comparison may be made.

Practically speaking, the changes noted in the brain above the level of the medulla are those of congestion, edema and hemorrhage. From the medulla downward through the cord there is increasing cellular infiltration and cuffing, as well as increasing degenerative changes, including actual necrosis of the motor neurons. This is true for both epidemics and is typical of the changes found in acute anterior poliomyelitis. In the 1934 epidemic there were apparently many cases in which the lesions

10. Landon, J. F., and Smith, L. W.: Poliomyelitis, New York, The Macmillan Company, 1934.

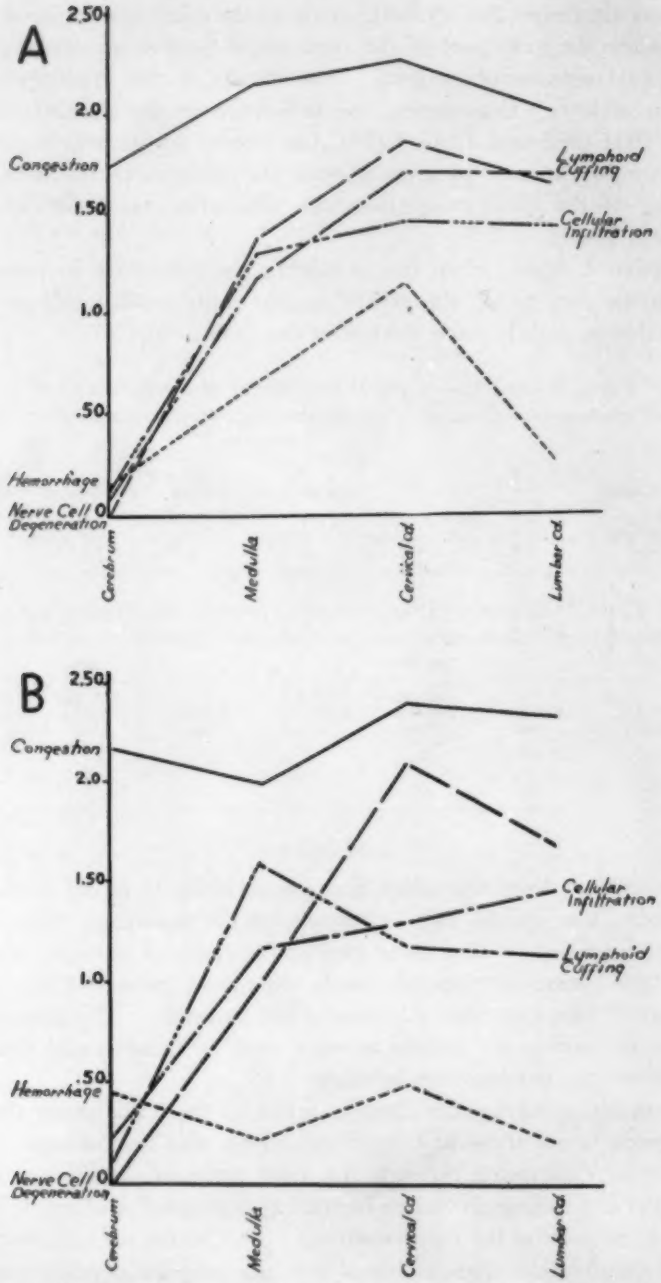

Fig. 3.—Graphs showing relative degrees of congestion, hemorrhage, cellular infiltration, lymphoid cuffing and cell degeneration at four levels in the central nervous system. *A*, 1934-1935 series; *B*, 1939 series.

were limited to relatively early inflammatory changes. There were greater infectiousness and greater communicability of the virus than are usual in this disease. When these strains of virus were injected into monkeys, they produced mild symptoms and a correspondingly low death rate (Kessel, Hoyt and Fisk [11]). In the patients who died the changes were characteristic of acute poliomyelitis, as has been demonstrated. Motor cell destruction was somewhat less severe in the cord in this epidemic than in the 1939 series. It seems probable that in the 1934 epidemic strains of poliomyelitis were present which were very infectious and highly communicable but less virulent, i. e., less destructive of motor neurons, than those usually encountered.

Although the records show that the majority of patients here reported died of bulbar palsy, this is not strictly correct, as McKhann [12] has pointed out. Many patients died of (1) direct paralysis of the muscles of respiration innervated from the dorsal and the cervical part of the cord (e. g., intercostal muscles and diaphragm); others died of (2) pharyngeal paralysis, which prevents swallowing, and allows secretions to accumulate in the glottis; others, of (3) involvement of the respiratory center. Only conditions 2 and 3 may be properly referred to as bulbar paralysis. The Drinker respirator is most useful in the group with failure of the muscles of respiration.

From the experimental, neurologic and pathologic standpoints it would seem that the much discussed 1934 epidemic in Los Angeles County fits into the scheme of only one disease, epidemic poliomyelitis.

SUMMARY

In spite of the unusual clinical manifestations of the 1934 epidemic of poliomyelitis in Los Angeles County, the pathologic changes in the central nervous system were typical of the disease.

The severity of the lesions in the spinal cord was somewhat less than is usually encountered in this disease and corresponds with the low incidence of paralysis and the low mortality rate of 1.39 per cent.

The lesions in the central nervous system in the 1939 epidemic were typical of epidemic poliomyelitis and were somewhat more destructive than those seen in 1934 and 1935. The clinical manifestations were mainly of the usual kind.

3551 University Avenue.

10431 Bellagio Road, West Los Angeles.

401 Bailey Street.

11. Kessel, J. F.; Hoyt, A. P. S., and Fisk, L.: Am. J. Pub. Health **34**:1215, 1934.

12. McKhann, C. F.: The Clinical Features and Treatment of Poliomyelitis, in Virus and Rickettsial Diseases with Especial Consideration of Their Public Health Significance: Symposium, Cambridge, Mass., Harvard University Press, 1940, p. 581.

CHANGES IN RETENTION OF COPPER AND IRON IN LIVER AND SPLEEN IN CHRONIC DISEASES ACCOMPANIED BY SECONDARY ANEMIA

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In secondary anemia the metabolism of copper and iron shows definite changes. Since the liver and the spleen are known to be storage centers for copper and iron, a study was made of the copper and iron content of each of these organs in various diseases accompanied by secondary anemia. Disturbances occur in the metabolism of copper and iron during the development of cancer. Iron is stored in the liver but can no longer be utilized normally. Copper is retained in the tumor cells, thus depriving the organism of copper. The lack of available copper and iron restricts the formation of erythrocytes, and secondary anemia results. In order to determine whether a relation exists between the changes in the copper and the iron content of the liver and the spleen and the character of the primary disease, cancerous as well as other diseases accompanied by secondary anemia were studied. Since the published data on the copper and the iron content of the liver and the spleen show considerable variation, we established our normal values by analyzing a series of presumably normal organs obtained from the medical examiner's office at Bellevue Hospital.

Gerlach [1] found that benign tumors had a normal copper content, whereas malignant tumors contained variable amounts of copper; mammary, esophageal, bronchial and intestinal tumors showed low and uterine and prostatic tumors high amounts. The copper content of the liver, however, was rarely changed. Edlbacher and Gerlach [2] stated that in Jensen sarcoma the copper content differs according to the part of the tumor studied. Necrotic tissue contains more copper than growing tumor. They found that the ratio between the copper content of necrotic tissue and that of tumor tissue remains unchanged though absolute values

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1. Gerlach, W.: Ztschr. f. Krebsforsch. **42**:290, 1935.

2. Edlbacher, S., and Gerlach, W.: Ztschr. f. Krebsforsch. **42**:272, 1935.

vary. Sümegi [3] studied copper in experimental rat cancers. He found that copper is deposited in the liver and the tumor and excluded from metabolism. The stomach contained 40 per cent less copper than normal, so that there is interference with formation of the antianemic factor. Bence [4] observed that while no parallelism exists between the copper and the iron content of the liver, a high iron content usually means an increase in copper storage too.

OBSERVATIONS IN ONE HUNDRED AND FORTY-SIX CASES

We present the results of a study of the copper and the iron content of the liver and the spleen in 146 cases of disease accompanied by secondary anemia (table). The primary diseases comprised cancer of the alimentary tract (38 cases), genito-urinary tract (13 cases), female pelvis (12 cases), breast (14 cases) and respiratory tract (11 cases); a lymphoblastoma group (16 cases); noncancerous diseases (41 cases).

Copper was determined according to the method of Elvehjem and Lindow [5] with slight modifications; iron, according to the modified method of Elvehjem and Hart.[6] All values are expressed as milligrams in 100 Gm. of dry tissue.

Cancer of the Alimentary Tract.—There were 15 cases in which the cancer involved the stomach. In 1 case the tumor was localized and caused pyloric obstruction; the figures for copper and iron in the liver and the spleen were normal. In another, with extension to the ovary alone, excess copper was found in the spleen, 1.6 mg. per hundred grams. In a third, with extension to gastric lymph nodes, the values for the copper and iron of the liver and the spleen were normal. In all the other cases there was greater involvement of adjacent or distant organs, and the figures for both elements were significantly higher. However, in an instance of colloid carcinoma of the stomach with metastasis to preportal lymph nodes and to the skin, analysis yielded values of 437 mg. of iron and 1.7 mg. of copper for the spleen; those for the liver, while high, were within the normal range. Evidently, marked retention was found with a relatively slow-growing tumor that had extended only to lymph glands. In general, however, extensive metastasis meant marked retention of both copper and iron.

The highest figure for iron in the liver, 255 mg. per hundred grams, was encountered in a case with widespread abdominal metastases. Marked

3. Sümegi, S.: Frankfurt. Ztschr. f. Path. **48**:35, 1935.
4. Bence, J.: Ztschr. f. klin. Med. **126**:143, 1933.
5. Elvehjem, C. A., and Lindow, C. W.: J. Biol. Chem. **81**:435, 1929.
6. Elvehjem, C. A., and Hart, E. B.: J. Biol. Chem. **67**:43, 1926.

Iron and Copper Contents of Liver and Spleen in Cases of Disease Accompanied by Secondary Anemia

Disease	Cases Examined	Liver		Spleen	
		Iron *	Copper *	Iron *	Copper *
Normal controls	15	17.2-81.4 (44.7)	1.4-4.5 (2.8)	49.8-252.3 (137.2)	0.7-1.2 (0.9)
Carcinoma of stomach	15	6-255 (70.1)	0.7-3.8 (2.3)	23-720 (223)	0.5-1.7 (1.2)
Carcinoma of pancreas	2	53-253 (153)	1.2-2.3 (1.8)	56-328 (192)	1.0-1.5 (1.1)
Carcinoma of duodenum	1	166	4.2	76	1.3
Carcinoma of esophagus					
With metastasis	1	207	3.0	715	1.6
Without metastasis	1	46	2.5	159	2.4
Carcinoma of rectum	12	7-458 (87)	2.1-6.4 (3.6)	75-2,146 (405)	0.8-3.3 (1.8)
Carcinoma of colon	4	27-37 (32)	2.6-3.4 (3.0)	82-326 (200)	1.5-3.1 (2.0)
Carcinoma of palate	1	116	2.9	20	4.0
Carcinoma of gallbladder	1	44	1.9	151	1.1
Carcinoma of lung	6	59-216 (123)	1.5-4.4 (3.0)	156-1,695 (570)	0.5-2.0 (1.3)
Sarcoma of lung	1	60	2.4	163	0.8
Carcinoma of bronchus	4	76-322 (178)	3.5-4.9 (4.4)	255-446 (333)	0.9-3.6 (2.1)
Carcinoma of prostate	1	21	2.3	96	1.6
Carcinoma of kidney	8	36-162 (70)	3.1-6.5 (4.3)	106-2,844 (624)	0.8-1.9 (1.4)
Carcinoma of bladder					
With metastasis	2	51-174 (113)	1.9-3.4 (2.7)	228-529 (379)	1.0-2.9 (2.0)
Without metastasis	2	7-82 (45)	4.0-5.1 (4.6)	19-20 (20)	2.3-2.4 (2.4)
Carcinoma of breast	14	37-579 (150)	0.5-39.5 (6.1)	44-2,505 (570)	0.2-10.7 (2.3)
Carcinoma of ovaries	7	21-1,133 (204)	1.2-6.4 (3)	74-1,254 (380)	1.1-6.3 (2.6)
Carcinoma of uterus	1	41	3.2	179	1.7
Carcinoma of cervix					
Without metastasis	2	21-64 (43)	1.3	42-302 (172)	0.8
With metastasis	1	22	4.1	440	1.5
Carcinoma of vulva	1	53	2.8	440	0.5
Sarcoma					
Without metastasis	1	32-161 (69)	1.0-5.9 (6.2)	154 791 (357)	0.9-1.7 (1.2)
With metastasis	6				
Hodgkin's disease	6	54-580 (162)	2.3-9.2 (5.8)	140-1,776 (711)	1.7-2.2 (1.9)
Myelogenous leukemia					
Involvement of other organs	2	53-483 (268)	3.0-5.0 (4.0)	108-133 (121)	1.1-1.6 (1.4)
No involvement of other organs	1	20	10.2	72	1.0
Uremia	5	5-127 (72)	2.0-4.0 (3.1)	40-548 (286)	1.2-2.7 (1.1)
General arteriosclerosis					
With cachexia	6	36-181 (93)	1.7-21.2 (6.7)	114-802 (322)	1.4-3.3 (2.1)
Without cachexia	4	6-81 (52)	1.3-8.3 (3.5)	86-269 (178)	0.4-1.1 (0.9)
Diabetes	6	58-178 (126)	0.9-3.4 (1.8)	100-555 (267)	0.5-3.0 (1.4)
Dystonia musculorum deformans	1	59	3.5	147	1.1
Craniotomy for tumor and abscess of the brain	1	39	2.6	148	1.7
Multiple sclerosis	2	51-222	1.2-8.1	336-339	1.6-3.5
Duodenal ulcer	1	12	2.0	35	2.7
Ulcerative colitis	2	11-35	3.6-3.9	120-244	1.3-1.8
Paget's disease of bone	2	19-77	2.9	34-163	1.4
Aplastic anemia	1	196	4.0	302	1.6
Cooley's anemia	1	400	...	97	...
Hemophilia	1	20	9.6	96	2.4
Agranulocytic angina	1	81	3.0	215	0.9
Gaucher's disease	1	237	5.7	..	...
Mycosis fungoides	1	49	1.9	97	0.9
Subacute bacterial endocarditis	4	35-116 (50.3)	...	43-242 (110.8)	...
Postoperative meningioma of spinal cord	1	0 (fatty)	...	33	...
Polycythemia vera	1	362	2.9	390	1.2

* Figures represent milligrams in 100 Gm. of dry tissue (averages in parentheses).

retention in the liver frequently meant similar retention of iron in the spleen, though there was no exact relationship. Significant retention of iron in the spleen did not necessarily mean similar retention of iron in the liver.

The highest figure for iron in the spleen was 720 mg. per hundred grams in a case with metastases in the abdomen and the chest. In that instance, however, the iron in the liver was within normal limits. Inversely, the liver contained a very high amount of copper, 3.8 mg. per hundred grams, while the copper content of the spleen was normal.

The greatest retention of copper in the spleen, 1.7 mg. per hundred grams, was recorded in 3 cases with extensive metastases. In 2 of these, the copper and the iron in the liver and the spleen were significantly increased, while in the third case the values were normal.

However, low or even normal figures for iron in the liver were found associated with marked retention of iron in the spleen or vice versa. Furthermore, the figures for copper in the liver and the spleen were frequently normal, while those for iron in the liver and the spleen were abnormally high. At times only one value was pathologic. Inspection of the figures for copper shows that storage of copper was less easily affected than storage of iron. When the copper level in the spleen was elevated, there was usually increased retention of iron in either the liver or the spleen.

In cases of gastric cancer the average iron content of the liver was 70.1 mg. per hundred grams of dry tissue and that of the spleen 223 mg., a definite increase above normal. The copper content of each of these organs was normal. In this group the only instances in which all figures were normal was one in which the cancer was localized to the stomach with infiltration of the pylorus and another with metastasis limited to gastric lymph nodes. Anemia alone could not be the determining factor, since it occurred in all cases. The only difference was that more extensive metastatic disease was present in those cases in which abnormal values were found.

Of 2 cases of cancer of the pancreas, there was increased retention of iron in the liver in the first, and of copper in the liver and the spleen in the second. There was, however, no relation between the height of the retention and the diffuseness of the metastatic spread. One case of carcinoma of the duodenum was accompanied by increased retention of iron in the liver and of copper in the spleen.

Of 2 cases of carcinoma of the esophagus, the one with metastasis showed marked retention of iron in the liver and the spleen, and of copper in the spleen. In the other one, in which no metastatic disease occurred but there was severe cachexia, the only increase was that of copper in the spleen.

Twelve cases of carcinoma of the rectum were analyzed; in 2 cases primary cancer occurred elsewhere as well; in the first of these there were colloid adenocarcinoma of the sigmoid colon with perforation into the cecum and primary papillary adenocarcinoma of the rectum. There was local limitation of the tumors, with perforation of one into the cecum, but no widespread metastasis. In the second case, with a primary cancer in the rectum and another in the kidney and microscopic metastasis to the bladder, only copper in the spleen was increased. In this entire group the averages were high. Furthermore, while one of the values might be normal, there was no single case in which there was no increased retention of copper or iron in the liver or the spleen. Values as high as 458 mg. per hundred grams for iron in the liver, 2,146 mg. for iron in the spleen, 6.4 mg. for copper in the liver and 3.3 mg. for copper in the spleen were obtained. High figures were especially common in the presence of severe anemia, but an exact or causal relation between the height of the figures and the severity of the anemia could not be established.

There were 4 cases of primary carcinoma of the colon, all with metastases. Marked increases in the copper or the iron in the liver or the spleen were present. A palatal carcinoma with diffuse local extension was associated with significant retention of iron in the liver and of copper in the spleen.

Cancer of the Respiratory Tract.—There were 11 cases of cancer of the lung, 6 of carcinoma and 1 of sarcoma of the lung, and 4 of bronchial carcinoma. In 1 case of carcinoma of the lung there was also a primary hypernephroma of the kidney. Two of the pulmonary cancers had no metastases. Both these tumors were huge. One was associated with extreme retention of iron in the liver and the spleen, while with the other there was a mild increase of copper in the spleen and of iron in the liver. The patient with marked retention of iron in the liver and the spleen was in a poor nutritional state, and this may have been a contributory factor causing retention. Evidently a huge tumor even in the absence of metastatic disease caused marked retention of both copper and iron. In this group, too, one of the values for copper or iron in the liver or the spleen might be normal, but the storage of at least one of the elements in one organ or the other was always affected. A patient with bronchial carcinoma with good nutrition and no metastases had abnormal retention of copper in the liver and the spleen and of iron in the spleen.

Cancer of the Genitourinary Tract.—Eight cases of cancer of the kidney were analyzed—7 of hypernephroma and 1 of sarcoma. In an instance of hypernephroma with an extension along the inferior vena cava there was 2,844 mg. of iron per hundred grams in the spleen. In another case the liver yielded 86 mg. of iron per hundred grams, but the

copper content of this liver was 6.5 mg. per hundred grams, an enormously high figure.

There were 4 cases of carcinoma of the urinary bladder. The condition of all 4 patients was poor. If one considers all the cases together, there was an increase in one or more values, whether or not metastasis was present. The figures, however, in the cases of metastatic carcinoma were very much higher. The copper in the liver and the spleen in the cases without metastasis was increased, and the iron in one liver was normal. In the cases with metastasis, the iron reached 174 mg. per hundred grams in the liver and 529 mg. per hundred grams in the spleen. The accompanying table shows significantly more iron in the liver and the spleen in the cases with metastasis than in the cases in which the cancers were localized and less copper in the cases with metastasis than in cases of localized cancer. The single case of primary prostatic carcinoma with local invasion of the bladder showed only increased retention of copper in the spleen. Evidently, primary cancer is associated with increased storage of copper and iron in the liver and the spleen. In the presence of metastases the values are enormously increased. Anemia, cachexia and dehydration may play a role. However, in the presence of cachexia and anemia the cases with metastasis in addition show values not seen in cases with cachexia alone. Metastasis appears in itself to be a primary factor determining increases in the copper and the iron content of the liver and the spleen, out of proportion to and possibly independent of the anemia.

Mammary Cancer.—The 14 patients with carcinoma of the breast all had metastases and varying degrees of secondary anemia. In some the degree of anemia was, however, not marked. Others were in a good state of nutrition or even showed obesity. The range of retention was extreme. There was no relation between the degree of anemia and the degree of retention of iron and copper in the liver and the spleen. The single point of note was the degree of retention and the extent of metastasis. In 1 case with extensive metastasis, we recorded the enormous figures of 2,505 mg. per hundred grams for iron in the spleen and 579 mg. per hundred grams for iron in the liver. In another instance the value for copper in the liver was 39.5 mg. per hundred grams. In this same case, however, only 1.6 mg. of copper per hundred grams was retained in the spleen. In general, when one value was increased, the others did not necessarily show marked retention. There were cases of extensive metastasis with only one value elevated. Increase in all four values did not indicate more widespread metastatic disease or poorer nutrition. Increase of iron in the liver did not mean increase of iron in the spleen, nor any increase of copper in either liver or spleen. When metastasis was present, retention was usually more severe.

Cancer of the Female Pelvis.—Twelve cases of cancer of the female pelvis were analyzed. These comprised 7 cases of ovarian cancer, 1 of uterine, 3 of cervical and 1 of vulvar cancer. In 2 cases of cancer of the cervix, no neoplasm was found at necropsy after treatment, including operation, and death was due to other causes. In the first of these cases figures were available for iron only and were normal. In the second case, in which there had been operative intervention, no cancer was found at necropsy, and death was due to hypertension and cardiac decompensation. The nutrition was good. The iron in the spleen was increased to 302 mg. per hundred grams, while all other values were normal. All the other cases showed extensive metastasis. Retention was marked, reaching as high as 1,133 mg. and 1,254 mg. of iron, and 6.4 mg. and 6.3 mg. of copper, per hundred grams in liver and spleen, respectively. The accompanying table shows that a cancer of the cervix without metastasis might be associated with normal or almost normal figures, but that with metastasis retention was marked.

Sarcoma.—Seven cases of various types of sarcoma were analyzed, including a case of primary sarcoma of the spleen. In the latter there was no metastasis but there was increased retention of copper and iron in the spleen. The cases with metastasis had the highest retention values. Marked cachexia prevailed in the majority of these cases and was present to at least a moderate degree in all of them.

Hodgkin's Disease.—Six instances of diffuse Hodgkin's disease were analyzed. Copper and iron retention was extremely high, and some of the highest values in the entire series were encountered in this group. Anemia was severe in all these cases, and in some the hemoglobin content was as low as 25 per cent or less. Nutrition was, however, good in at least 3 of the 6 cases.

Leukemia.—There were 3 cases of myelogenous leukemia. In 1 instance there was generalized lymphatic involvement. In another, bone marrow and abdominal organs were affected. In the case of myelogenous leukemia in the exhaustive phase with enlargement of the spleen there was much less marked retention of iron in the liver and the spleen, but the copper content of the liver was 10.2 mg. per hundred grams. The case in which there was the least involvement by myelogenous leukemia there was the most marked anemia. The retention figures were, however, much smaller than in the other 2 cases.

Uremia.—There were 41 purely medical cases. In 5 cases there was uremia—in 2 from arteriosclerosis and in 3 from glomerulonephritis; severe anemia was present in all, with the hemoglobin content ranging from 31 to 40 per cent and red cell counts from 1,500,000 to 2,500,000; also present were cardiac enlargement and hypertension. Significant retention of iron took place in 3 cases. Retention of copper in the

spleen occurred in 2 cases, but there was no increased retention of copper in the liver. Despite the anemia, the values do not approximate those encountered in cases of cancer, especially those with metastases.

Subacute Bacterial Endocarditis.—Four cases of subacute bacterial endocarditis were analyzed. All were complicated by rheumatic heart disease and uremia, and 1 showed in addition a congenital cardiac lesion, patent foramen ovale. In 2 there were emaciation and severe anemia, and in 1 nutrition was poor; in the fourth, however, there was marked obesity. Figures were available for iron only, and these were essentially normal. In the case with obesity there was a fatty liver which contained no iron. Iron in the spleen was low.

Arteriosclerosis.—Ten cases of diffuse arteriosclerosis were studied. Eight of the patients had marked cardiac enlargement or hypertension or both, with severe arteriosclerotic heart disease; 1 had paralysis agitans and another hemiplegia resulting from cerebral arteriosclerosis. Emaciation was moderate or marked in 6 patients; 3 patients were well nourished, and 1 was obese.

If one divides these cases according to the presence of emaciation or its absence the following variations are to be seen:

It is evident that in arteriosclerosis with cachexia the degree of retention was significantly increased above normal and single values were very high in some cases. The averages of all values indicated increased retention and were higher than in cases with good nutrition, in which the average and the individual figures were normal except for sporadic increases. Iron in the spleen amounting to 802 mg. per hundred grams and copper in the liver as high as 21.2 mg. per hundred grams were encountered in the cachectic group. These figures represent very great retention. Three patients without cachexia had chronic coronary disease and myocardial damage and 1 had chronic tuberculosis of the pleura and the pericardium with hemiplegia. All had anemia as well. The levels were essentially those observed in normal tissue.

Diabetes.—Determinations were available in 6 cases of diabetes. Anemia was present in 5, and there was significant retention in all. There was 1 instance in which anemia and emaciation was severe. It is interesting that in this case the iron in the spleen amounted to 555 mg. per hundred grams, the highest in the entire group. The cases in which nutrition was good showed slightly increased or normal values for iron in the liver. An interesting comparison may be drawn between the cases in which poor nutrition and anemia were shown and 1 in which the hemoglobin value and the red cell count were only slightly reduced. In the latter case the figure for hepatic iron was somewhat increased but not nearly as much as in the cases with severe anemia.

The diabetic patients had various associated diseases: hypertension, coronary disease, congestive heart failure and peripheral vascular disease. In only 1 was there manifest emaciation. Nevertheless, here again retention was slight and usually limited to iron.

Miscellaneous Conditions.—In a case of dystonia musculorum deformans with emaciation the values for copper and iron were normal.

In a case of tumor of the brain with abscess and poor nutrition there was retention of copper in the spleen only.

Two cases of multiple sclerosis—one with emaciation, the other with good nutrition—showed increased retention, more marked in the case with emaciation. Figures for hemoglobin were not available in either.

In a case of postoperative meningioma of the spinal cord with decubital ulcers and marked secondary anemia, figures were available for iron alone. The liver was fatty and showed none. Iron in the spleen amounted to only 33 mg. per hundred grams, showing that even with anemia there may be no increased retention.

A case of duodenal ulcer with anemia and emaciation showed retention of copper in the spleen and low values for iron in the liver and the spleen.

Two cases of ulcerative colitis in which emaciation and anemia occurred showed normal retention except for a somewhat higher storage of copper in the spleen.

Two cases of Paget's disease of bone with arteriosclerosis, cardiac enlargement and congestive heart failure were also found to show normal values.

One instance of aplastic anemia and another of erythroblastic (Cooley's) anemia were studied. The figures for hemoglobin and red cells were low, those for hemoglobin reaching 12 per cent in the case of aplastic anemia. There was marked retention of iron in the spleen and the liver and of copper in the spleen. The value for copper in the liver was at the upper normal limit. In the case of Cooley's anemia there was marked retention of iron in the liver but not in the spleen.

One case each of agranulocytic angina and another of hemophilia were studied. The hemophilic patient was emaciated; the one with agranulocytic angina was not. In the case of hemophilia there was a high retention of copper in the liver and the spleen while in the case of agranulocytic angina the values were normal.

A case of polycythemia vera showed retention of iron only in the liver and spleen.

In an instance of Gaucher's disease with anemia there was increased retention of iron and copper in the liver.

In a case of mycosis fungoides with poor nutrition and anemia the values were normal.

COMMENT

Since normal formation of hemoglobin is dependent on adequate stores of copper and iron, one would expect a decreased retention of these elements in anemia. However, in a study of copper and iron storage in severe chronic diseases accompanied by secondary anemia we found that not only is there no inadequate retention of these metals but even larger than normal amounts are stored in the depot organs, the liver and the spleen. Since we observed these large accumulations of copper and iron in many chronic diseases and especially in association with cancer, it seems that a deficiency of copper or iron or both cannot be the prime or the sole determining factor responsible for anemia. It seems rather that the power to synthesize the blood-forming elements is impaired. Under such circumstances large stores of copper and iron, presumably retained for emergency purposes, can no longer function in the conversion of inorganic iron into hemoglobin, which may explain the paradox of the excess of copper and iron in the liver and the spleen, on the one hand, and severe anemia, on the other. Additional factors may be toxic disturbances in protein metabolism or loss of large amounts of protein. No definite correlation could be found between the degree of nutrition and the retention of either copper or iron except that increased retention was usually present in either the liver or the spleen in poor nutrition or anemia. There was no rule as to which element would be retained or, if both were retained, which would be stored to a greater extent, though retention of iron was more prevalent and usually greater than that of copper. An outstanding feature, however, was the tenacity with which the liver maintained its copper content, irrespective of iron retention. This fact has been noted by many investigators. The accompanying table shows that the liver seems to guard its storage of copper, in most instances, regardless of the amount of copper present in the spleen, or of iron in the liver and the spleen.

While anemia is of major importance in increased storage of copper and iron, the extent of storage is out of all proportion to the anemia in cases of cancer and reaches its highest point in cases with extensive metastasis. Curiously enough, enormous amounts of copper and iron were found in several cases of cancer in which there was no anemia, showing that such accumulation may occur even in the absence of anemia, with cancer as the only apparent causative factor. In some cases, by an analysis of liver and spleen alone it was possible to predict whether cancer would be found. What factor is responsible for the excessively high copper and iron contents in the liver and the spleen in cases of malignant tumor—whether accompanied by anemia or not—is not yet clear. The huge accumulation of these metals is, however, so striking and constant that it must be considered significant.

Since the formation of hemoglobin and the maturation of blood cells are possible only in a suitable physicochemical environment, it seems conceivable that disturbances in the oxidation reduction potential, sulfhydryl metabolism and enzymatic activation make the large deposits of copper and iron unavailable.

SUMMARY

A study has been made of the changes in copper and iron storage in the liver and the spleen in 150 cases of severe chronic disease accompanied by secondary anemia.

In chronic diseases of various types accompanied by anemia huge stores of copper and iron may accumulate in the depot organs.

In cancer accompanied by anemia the marked increase in both copper and iron storage was out of all proportion to the anemia. In some cases of cancer, such increases took place even in the absence of anemia, with cancer as the only apparent causative factor. The excessive copper and iron retention in the liver and the spleen encountered in cases of cancer is significantly higher in cases with extensive metastasis.

There was no rule as to which element would be retained or, if both were retained, which would be stored to a greater extent. Retention of iron was more prevalent and usually greater than that of copper. The liver appeared to maintain a close guard over copper storage; abnormal values were found in only 16 per cent of our cases.

EFFECT OF WEIGHT ON THE DEVELOPMENT OF MAMMARY CARCINOMA IN VARIOUS STRAINS OF MICE

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In a series of papers, we [1] have analyzed the relation between age and the incidence of carcinoma of the mammary gland in various strains of mice. In continuing these investigations with other strains, we began several years ago to make some experiments in which we underfed mice in order to determine whether underfeeding lowers the number of tumors developing in these strains. While there was some indication in these experiments that there might be such an effect of the nutritional state of the mice on tumor development, the number of experiments was too small for definite conclusions to be drawn. We decided then to study by statistical means the relation between the weights of the mice observed by us and the growth of the mammary glands and ultimately the end stage of the growth process, consisting in the cancerous transformation of these mammary glands. In this transformation the activity of a virus may, perhaps, be involved in addition to the preparatory growth processes.

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These investigations were carried out with the aid of grants from the International Cancer Research Foundation and from the Jane Coffin Childs Memorial Fund for Medical Research. Alpha estradiol benzoate in oil (progynon B—Schering) was the estrogen used in our experiments. Progesterone in oil (proluton) and, in some experiments, a corpus luteum extract prepared in our laboratory were the sources of the luteal hormones. Dr. Erwin Schwenk supplied these preparations.

1. (*a*) Lathrop, A. E. C., and Loeb, L.: J. Exper. Med. **22**:646 and 713, 1915. (*b*) Loeb, L.: J. Cancer Research **6**:197, 1921. (*c*) Suntzeff, V.; Kirtz, M. M.; Blumenthal, H. T., and Loeb, L.: Cancer Research **1**:446, 1941. (*d*) Tureen, L. L., and Loeb, L.: J. Cancer Research **13**:1, 1929.

WEIGHTS OF NORMAL MICE OF DIFFERENT AGES

Before analyzing the relation between the average weight of the mice and the growth of the mammary glands and the cancerous transformation of these glands, it will be necessary to determine the average weights of normal mice in various age groups in various strains. For this purpose the average weight of 10 or more mice belonging to each age level in each of the various strains was determined. In cases in which a smaller number of mice was used, this number is indicated in table 1 in brackets. The weights of at least 643 male mice, of approximately 608 female virgin mice and of approximately 340 breeding female mice were thus determined; at least 1,591 normal mice were weighed at various ages.

From the figures given in table 1 it may be concluded: 1. The weight of mice increases rapidly (from 70 to 100 per cent) between the age of 2 weeks and 1 month; after this age the increase in weight slows down more and more, and the maximum is reached between the ages of 9 and 11 months. In still older mice certain variations occur, but the maximum weight remains about steady until after the age of 20 months. These figures apply equally to male, to virgin female and to breeding female mice.

2. The average weight of male mice is greater than that of female virgin mice; this holds good in the case of all age groups, and, furthermore, the difference is of a similar order in all age groups. The weights of breeding females were determined from the age of 4 months on; they were similar to those noted in males. The average weight for all male mice from this age on was 26.1 Gm., that for virgin female mice was 22.6 Gm. and that for breeding female mice was 25.3 Gm.

3. If the average weights of all age classes in the various strains are compared, males, virgin females and breeding females of strain Old Buffalo are found to be the heaviest. In the group of male mice, the New Buffalo strain is next. Strains C57, C3H, CBA and A follow, without any great difference being noticeable between these strains. Strain D has the lowest weight.

Among the virgin female mice, the Old Buffalo strain has, as stated, the greatest weight; strains C57, A and New Buffalo come next, while strains C3H, CBA and D have the lowest weights. In the breeding female mice, strain Old Buffalo is followed by strains C57, C3H, New Buffalo and A. Strains D, CBA and AKA have the lowest weights. In general, it may be concluded that strain Old Buffalo is heaviest; that New Buffalo and C57 follow and that D has the lowest weight. Strains C3H, A and CBA differ somewhat in their position in these three groups.

4. More definite results are obtained if we consider separately mice from 1 to 3 months old and mice 4 months old and older, as in table 2.

TABLE 1.—*Average Weights (in Grams) of Normal Mice at Different Age Levels in Various Strains*

Strain	0.5 Mo.	1 Mo.	2 Mo.	3 Mo.	4-8 Mo.	9-11 Mo.	13-16 Mo.	17-20 Mo.	Over 20 Mo.	Av. Weight According to Strain *
Males †										
Old Buffalo	.....	20.7	21.5	24.8	26.4	26.8	29.8	31.7	32.2	26.7
New Buffalo	.....	22.5	21.3	19.7	24.5	30.5	25.5	27.5	28.5	25.0
C57	7.2	15.4	21.6	23.0	26.6	28.7	24.9	25.5	28.5 (3)	23.9
C3H	13.0	15.0	19.9	22.9	24.3	27.6	26.3	27.0	28.0	23.9
CBA	.....	8.0	20.6	25.5	24.1	25.6	26.5	24.0	27.6	22.7
A	.....	15.3	17.0	23.6	24.3	26.9	25.1	25.0	25.7	22.9
AKA	.....	.....	.....	24.8	23.1	26.1	22.3	24.5	26.0	.....
D	6.6	12.7	17.1	17.8	23.5	24.2	20.3	23.6	21.0	20.0
Av. weight according to age...	8.9	15.7	19.8	22.8	24.6	27.0	25.1	26.1	27.1	
Number of mice—643										
Virgin Females †										
Old Buffalo	.....	11.5	19.4 (4)	.....	22.4	26.5	23.0	28.2	32.7	23.8
New Buffalo	.....	13.0	18.7	18.8	24.6	22.0	19.8	21.0 (2)	23.0 (2)	19.6
C57	6.7	15.7	17.4	20.6	21.1	20.0	25.4	24.1	29.0	21.7
C3H	.....	8.3	14.4	14.7	18.6	21.9	20.7	19.3	.....	16.8
CBA	4.0	13.5	15.5	19.0	18.9	17.5	19.4	18.0	24.0	18.2
A	.....	13.3	18.2	19.2	20.5	25.2	22.7	22.9	24.0	20.7
AKA	.....	.....	21.0	25.2	22.0	23.4	23.8	24.5	22.0	23.1
D	6.8	11.8	18.7	18.1	20.0	20.3	17.2	18.3	.....	17.8
Av. weight according to age...	5.8	12.4	17.7	19.4	21.0	22.1	21.5	22.0	26.2	
Number of mice—608										
Breeding Females †										
Old Buffalo					28.9	28.5	28.5	28.7	33.2	29.6
New Buffalo					24.6	26.1	23.2	29.0	.....	25.7
C57					26.0	28.7	26.7	26.4	26.0	26.8
C3H					25.1	27.3	29.7	24.9 (4)	.....	27.1
CBA					22.3	26.7	25.8	19.9	21.0	23.1
A					25.5	26.2	27.5	18.5	23.0	24.1
AKA					21.3	20.5	24.5	24.3 (4)	.....	22.4
D					24.7	25.8	22.5	20.0 (2)	.....	24.1
Av. weight according to age					24.8	26.2	26.0	24.4	25.8	
Number of mice—340										
Total number of mice—1,591										

* The averages in this column do not include the weights of mice 0.5 month old.

† A figure in brackets represents the number of animals on which the determination of average weight is based. Every other determination is based on the weights of at least 10 mice.

Among the male mice, the order of the animals according to decreasing weight is the same in the younger and in the older age groups. It is as follows: Old Buffalo → New Buffalo → C57 → C3H → CBA and A → D. In the virgin female mice the order in the older age group is: Old Buffalo → C57 → New Buffalo → A = AKA → CBA = C3H → D. In the breeding female mice the order is: Old Buffalo → C57 → C3H → New Buffalo → A → CBA = D.

TABLE 2.—*Average Weights (in Grams) of Two Age Groups of Male, Virgin Female and Breeding Female Mice in the Various Strains of Mice*

Strain	1, 2 and 3 Mo. Old	4 to Over 20 Mo. Old
Male Mice		
Old Buffalo	22.3	29.4
New Buffalo	21.2	27.3
C57	20.0	25.8
C3H	19.3	26.6
CBA	18.0	25.6
A	18.6	25.4
AKA	.....	24.5
D	15.9	22.5
Virgin Female Mice		
Old Buffalo	.....	26.6
New Buffalo	16.8	22.1
C57	17.9	23.9
C3H	12.4	20.1
CBA	16.0	19.6
A	16.9	23.1
AKA	.....	23.1
D	16.2	18.9
Breeding Female Mice		
Old Buffalo		29.6
New Buffalo		25.7
C57		26.8
C3H		27.1
CBA		23.1
A		24.1
AKA		22.4
D		24.1

TABLE 3.—*Average Weights of All Mice in Two Age Groups in Various Strains*

Strain	1 to 4 Mo. Old	4 to Over 20 Mo. Old
Old Buffalo	22.3	28.3
New Buffalo	19.0	25.0
C57	18.9	25.8
C3H	15.8	24.4
CBA	17.1	22.8
A	17.75	24.2
AKA	.....	23.4
D	16.05	21.8

The averages of the various strains in both age groups without division into males and females are shown in table 3; as seen here, the following order applies approximately to both groups: Old Buffalo → C57 = New Buffalo → C3H = A = AKA = CBA → D.

WEIGHTS OF EXPERIMENTAL MICE

After having established the average weights of normal mice at different ages in the various strains as a standard, we shall now compare with these the weights of the mice to which injections of various quantities of an estrogen alone or in combination with a preparation of progesterone or an extract of corpus luteum were given; in addition, some mice had received anterior hypophysial transplants. The animals included in these statistics are identical with those which we have studied in preceding papers. However, it will be necessary first to determine the average weights of the mammary tumors (carcinoma) in order to obtain the net weights of the mice bearing the tumors. In table 4 are shown the weights of the tumors, increasing with increase in volume.

The weights of the tumors from the treated mice usually amounted to small fractions of 1 Gm. Also, the weights of the tumors from control

TABLE 4.—*Weights of Tumors of Various Sizes*

Diameters	Weights, Gm.
0.8 × 0.8 × 0.5 cm.	0.21
1.2 × 0.8 × 0.8 cm.	0.35
1.0 × 0.9 × 0.9 cm.	0.53
1.6 × 1.5 × 0.8 cm.	0.93
2.3 × 1.7 × 1.2 cm.	2.18
2.3 × 1.8 or 1.9 × 1.2 cm.	2.53
2.4 × 1.8 × 0.8 cm.	2.83

The weights of tumors from mice given injections usually amounted to small fractions of 1 Gm.

The weights of tumors from control mice were usually below 1 Gm., but in some cases may, perhaps, have reached a greater weight.

mice, as a rule, were below 1 Gm., but a few may have exceeded this figure.

In order to avoid some of the variable factors which might lead to erroneous conclusions, we have eliminated from our statistics all mice younger than 4 months, because the variations in the weights of these mice are greater than those of mice 4 months old or older. We have further omitted from among the records of the nontreated mice those of all males, because the mammary glands of these males did not show any growth. There remain altogether 1,008 mice which were used in our experiments; this includes also groups of animals not treated with glandular substances, with which the experimental mice could be compared and which served thus as special controls. In each of these groups we determined not only the average weight but also the average age because, as we have shown, age is a factor which may influence the weight of mice. The majority of the nontreated mice were virgin females, but among them were also some breeding female and some ovariectomized mice.

We shall first arrange the mice according to the grades attached to the state of proliferation of their mammary glands and then compare with these grades the weights of the animals, considering at the same time the ages of the latter.

TABLE 5.—*Relations Between Weight and Proliferative State of the Mammary Gland in Nontreated and Treated Mice Belonging to Various Strains*

Class	Group I (Grade 4)			Group II (Grades 2 and 3)			Group III (Grades 1—, 1, 1+)		
	Av. Weight, Gm.	Mice	Av. Age, Mo.	Av. Weight, Gm.	Mice	Av. Age, Mo.	Av. Weight, Gm.	Mice	Av. Age, Mo.
Strain C3H									
Nontreated females	30.8	21	12.1	27.5	2	16	.....	....	.....
Treated males.....	28.5	11	10.97	25.8	5	12.7	26.1	13	11.6
Treated females.....	28.6	21	9.9	18.1	14	6.6	21.7	9	5.5
Strain D									
Nontreated females	29.4	25	14	.....	....	.....	24.3	8	9.25
Treated males.....	23.6	5	8.4	23.9	4	6.1	22.4	6	12.8
Treated females.....	25.4	25	15.4	23.5	12	14.6	19.4	11	9.5
Strain A									
Nontreated females	31.3	7	13.4	31.0	3	16.7	26.7	43	9.6
Treated males.....	28.8	9	9.8	15.7	2*	11	25.6	9	8.7
Treated females.....	28.6	26	13.2	23.5	16	11.9	22.1	54	9.4
Strain New Buffalo									
Nontreated females	32.4	7	19	24.0	2	18	26.1	13	9.8
Treated males.....	.....	....	.....	24.5	3	8.1	18.6	6	7.3
Treated females.....	32.3	3	13.8	28.1	7	13	15.0	1	7
Strain Old Buffalo									
Nontreated females	42.7	3	20.4	.....	....	.....	29.6	34	9.4
Treated males.....	30.0	1	13.5	31.5	3	31.75	.....	....	.....
Treated females.....	33.3	3	12.4	21.7	5†	8.75	25.5	12	13.3
Strain CBA									
Nontreated females	25.4	6	18.8	24.5	3	13.3	25.0	17	15.8
Treated males.....	.....	....	.....	22.8	6	6.7	29.0	2	6
Treated females.....	.....	....	.....	24.9	18	7.9	25.6	7	7.6
Strain C57									
Nontreated females	.....	....	.....	.....	....	.....	23.8	48	8.2
Treated males.....	.....	....	.....	30.8	6	9.7	25.7	9	8.6
Treated females.....	.....	....	.....	30.1	14	12.8	25.2	43	10.3

* These mice were underfed.
† This number includes sick mice.

Table 5 shows the relation between the grades of proliferation of the mammary glands and the weights and ages of the seven strains of mice which are included in our statistics.

In each strain three classes are distinguished, namely (1) nontreated female mice, which did not receive any injections, (2) male mice which received injections of different kinds of glandular substance or combinations of such substances and (3) female mice treated with various combinations of these substances. Each of these classes is further subdivided according to the grades representing the state of proliferation of the mammary glands, and the following three groups are used: Group I, mice in which the growth of the mammary glands has progressed to the carcinomatous stage (grade 4); II, mice in which proliferation

of the mammary glands has advanced to the formation of lobules or larger groups of acini (grade 2) and to precancerous growth (grade 3), and III, mice in which proliferation has proceeded merely to the formation of a variable number of ducts, with or without the production of a limited number of isolated acini or of small groups of acini (grade 1). In each of these subdivisions the number of mice, the average weight and the average age of the mice are given. We thought it advisable to include also data as to the average age, although variations in age among mice older than 4 months as a rule affect the weight only to a moderate degree.

From the figures in table 5 it may be seen that in the nontreated mice the weights of the bearers of tumors are higher than those of the mice which did not have tumors. This is true when there is only one group of nontumor mice. Likewise, in those strains in which there are two groups of nontumor mice—namely (*a*) those with grades 3 and 2 and (*b*) those with grade 1—the average weight of the tumor mice is greater than that of the combined two groups of nontumor mice. However, in strain CBA the difference between these two figures is so small that it is of no significance; here the average weights of the tumor and the nontumor mice are approximately the same. This may be partly explained by the very small number of mice in the group with grade 4. For a similar reason, not much importance can be attached to the similarity in weight between mice with grade 4 (bearers of tumors) and those with grades 3 and 2 (nontumor mice) in strain A. In all the strains the difference between the groups with grade 4 (tumor mice) and those with grade 1 is quite definite except again in strain CBA. It is possible that in a strain with as low hereditary tendency to the development of carcinoma of the mammary gland as that possessed by strain CBA, other factors may preponderate to such a degree that the weight factor becomes negligible. In three strains, the average weights in group II (grades 2 and 3) and in group III (grade 1) are available for comparison; only in strain A is the average weight greater in group II than in group III. The difference in age of the mice in the various strains and groups is not of great significance. The conclusion may be drawn, then, that the average weight of the tumor mice in the nontreated groups is higher than that in the other groups and that this difference in weight exceeds the weight of the tumors.

Tables 1 and 2 have shown that mice belonging to strain Old Buffalo usually reach the greatest weight; they are followed by mice belonging to strains C57 and New Buffalo, while in strain D are the mice with the lowest weight. The data in table 5 agree with these findings. Old Buffalo and New Buffalo mice with tumors have a greater weight than mice belonging to the other strains, while again strain D mice have the lowest weight. However, there is one exception—in strain CBA the weight of the 6 tumor mice is lower than that of the strain D tumor mice, although the weight of the CBA nontumor mice is higher.

The male mice which received injections and in which tumors developed have a lower weight than the nontreated tumor-bearing mice. While the number of mice used in these experiments is small, it is again seen that the weight of the tumor mice is, on the average, somewhat higher than that of the nontumor mice with grades 3, 2 or 1 and that there is no consistent difference between the weights of the mice with grades 2 and 3 and the weights of those with grade 1.

Among the female mice treated with estrogenic, luteal or pituitary substances, it is again the Old and the New Buffalo tumor mice which have the greatest weight, while the D mice have the lowest weight. Here the greater weight of the tumor mice as compared with the nontumor mice is very evident in the various strains, while the difference between the weights of mice with grades 3 and 2 and the weights of those with grade 1 are variable.

These conclusions are confirmed in table 6, in which the combined averages of the weights and ages of the nontreated and the treated male and female mice with the various grades are given without relation to strains. Among the nontreated animals the weight of the bearers of tumors is somewhat greater than that of the nontumor mice. Among the treated mice the difference is very definite in males as well as in females. Among the treated males and females with grade 1 the weight is somewhat lower than that of the mice with grade 2 or 3, in contrast to the nontreated mice among which the weight of animals with grade 1 is somewhat higher than that of animals with grade 2 or 3. If we consider the average weights of these three classes of animals, the greater weight of the tumor mice is evident. Among the nontumor mice, the average weight of the mice with grade 1 is somewhat greater than that of the mice with grades 2 and 3. The average weight of the tumor mice is 29 Gm., that of mice with grades 3 and 2 is 24.8 Gm. and that of mice with grade 1 is 25.9 Gm. The average weight of the mice with grade 3 or 2 does not, therefore, exceed that of mice with grade 1.

Classes IV, V and VI represent various experiments in which the numbers of animals involved were rather small. To class IV belong 12 castrated male strain A mice which had received ovarian transplants. Class V comprises female ovariectomized or nonovariectomized mice which had received ovarian transplants; there were only 8 mice in this group, which in addition suffered from the fact that the tumor mice belonged to strains A and D, while the nontumor mice belonged to strain AKA. Thirty-four mice belonged to class VI, which consisted mostly of female mice bearing hypophysial transplants, with or without transplants of other endocrine organs. In this group, also, there was a lack of genetic homogeneity, the tumor mice belonging to strains A and D, while the nontumor mice belonged to strain C57. The combined classes of IV, V and VI show a greater average weight of the tumor mice than of the mice with grade 1, but the average weight of the mice with grade 3 or 2 exceeds the weight of the tumor mice. If we consider groups I to VI combined, the average weight of the tumor mice is notably greater than that of the nontumor mice (28.8 Gm., compared with 25.2 and 25.8 Gm.). The average weights of mice with grades 2 and 3 and mice with grade 1 are about the same. These figures are based on 682 mice altogether; some importance may therefore be attached to these differences in weight.

There are three additional groups of mice, which had been given injections of estrogens or of other hormones or hormone-like substances (an acid extract of the anterior lobe of the hypophysis, thyroxin or potassium iodide), which differ from the others in that the mice used were examined only grossly. In these classes only two groups can therefore be distinguished, namely, those which were bearers of tumors and those in which mammary tumors had not yet developed. No statement can be made concerning the degree of proliferation in the mammary glands in these classes. In every one the average weight of the tumor mice was definitely greater than that of the nontumor mice. The total average weight was 24.5 Gm. for the tumor mice and 21.1 Gm. for the nontumor mice. Sixty-two tumor mice and 264 nontumor mice were included in these statistics. The average age was about the same in these two groups, namely, 11 and 10.8 months. These results are confirmatory of those obtained in groups I to VI.

We shall now compare the average weights in the various age classes of mice which had received injections of estrogen, with or without the addition of corpus luteum extract, with the weights of the nontreated mice of various ages, as shown in table 1.

In table 7 are shown the average weights of the mice in the various age classes, which had been given injections of estrogen or of combinations of estrogen and luteal preparations. Excluded are mice below the age of 4 months. In order to

TABLE 6.—*Average Weight and Age of the Various Groups Without Regard to Strain (Mice 4 Months Old or Older)*

	With Tumors			Without Tumors					
	Grade 4			Grades 2 and 3			Grades 1−, 1, 1+		
	Av. Weight, Gm.	Mice	Av. Age, Mo.	Av. Weight, Gm.	Mice	Av. Age, Mo.	Av. Weight, Gm.	Mice	Av. Age, Mo.
I. Nontreated mice	30.7	69	14.8	26.9	10	15.8	29.5	144	11.1
II. Male mice given injections of estrogen, anterior hypophysial extract or extract of corpus luteum	27.7	26	10.2	25.7	29	9.1	22.5	49	8.97
III. Female ovariectomized and nonovariectomied mice given injections of estrogen, anterior hypophysial extract or extract of corpus luteum	27.9	78	13	24.3	86	10.6	23.3	137	6.8
Classes I, II and III combined	29.0	173	13.3	24.8	125	10.7	25.9	330	8.9
IV. Castrated male strain A mice with ovary transplants V. Ovariectomized and nonovariectomized female mice with ovary transplants VI Mostly female mice with anterior hypophysial transplants, with or without ovary, thyroid, testicle, adrenal or pancreas transplants	26.6 *	11	11.9	28.1	15	14.2	24.8	28	14.1
Classes I to VI inclusive	28.8	184	13.2	25.2	140	11.1	25.8	358	9.3
					Av. Weight, Gm.		Mice	Av. Age, Mo.	
VII. Mice belonging to strains D, C3H, A or CBA given injections of various substances	24.7	48	11.6		21.5		193	10.16	
VIII. Male C3H mice given injections of estrogen for 5 mo.	24.9	10	9.25		21.0		26	11.5	
IX. Male D mice given injections of estrogen for 5 mo.	22.1	4	10.7		20.6		45	13.2	
Classes VII, VIII and IX combined	24.5	62	11.2		21.1		264	10.8	

* The figures are for classes IV, V and VI combined.

TABLE 7.—*Average Weights of Mice at Various Age Levels Which Received Injections of Large and Small Doses of Estrogen or of Estrogen and Luteal Extracts*

Strain	4-5 Mo.		6-8 Mo.		4-8 Mo. *		9-11 Mo.		4-11 Mo. †		12-20 Mo.		21-25 Mo.	
	Av. Weight, Gm.	Mice	Av. Weight, Gm.	Mice	Av. Weight, Gm.	Mice	Av. Weight, Gm.	Mice	Av. Weight, Gm.	Mice	Av. Weight, Gm.	Mice	Av. Weight, Gm.	Mice
Old Buffalo	21.0	2	20.0	1	20.7	3	.....	....	.....	....	28.2	15	.....	..
New Buffalo	18.4	5	21.8	7	20.4	12	24.0	2	20.9	14	33.0	7	.....	..
C57	22.9	17	25.4	15	24.1	32	30.3	14	26.1	46	29.2	18	25.3	6
C3H	24.8	11	22.9	31	23.4	42	27.6	10	24.2	52	27.5	20	.....	..
CBA	21.5	9	27.6	14	25.1	23	24.8	9	25.1	32	21.0	1	.....	..
A	23.1	13	25.5	31	24.8	44	24.2	36	24.5	80	25.5	39	25.0	1
D	20.2	3	22.4	11	21.96	14	25.1	10	23.3	24	25.0	22	21.4	6
Total averages..	22.5	60	24.5	110	23.8	170	25.9	81	24.5	248	27.0	122	23.5	13

* This column (column 3) is a combination of columns 1 and 2.
† Column 5 is a combination of columns 3 and 4, except that the 3 Old Buffalo mice in column 3 are omitted from column 5.

facilitate the comparison there are given, in table 8, the average weights of normal male, virgin and breeding female control mice and the total averages of these three groups.

The averages of the weights in all the strains in control mice and in treated mice show a great similarity: from 4 to 8 months, 23.3 Gm. in the controls and 23.8 Gm. in the treated mice; from 9 to 11 months, 25.1 Gm. in the controls and 25.9 Gm. in the treated mice. There is some difference in the older mice—from 12 to 20 months: 24.2 Gm. in the controls and 27 Gm. in the treated mice, and in mice over 20 months, 26.2 Gm. in the controls and 23.5 Gm. in the treated mice. However, in the last-named age groups the numbers of available mice are relatively slight. Moreover, there are some variations in the weights of the different classes from the age of 11 months on in the controls. While in the controls the maximum weight is reached at the age of from 9 to 11 months, in the treated mice there is a further increase in weight in the period from 12 to 20 months.

As to the average weights of the mice belonging to the various strains which had been given injections, the number of Old Buffalo and New Buffalo mice is so small that they must be omitted. The average weights in the other strains are

TABLE 8.—*Averages of Weights (in Grams) in All the Strains of Control Mice*

Age	Males, Av. Weights	Virgin Females, Av. Weights	Breeding Females, Av. Weights	Total Average of All Three Classes
3 months	22.8	19.4	.....	21.1
4- 8 months	24.2	21.0	24.8	23.3
9-11 months	27.0	22.1	26.2	25.0
13-16 months	25.0	21.5	26.0	24.2
17-20 months	26.1	22.2	24.4	24.2
Over 20 months	27.1	25.8	25.8	26.2

approximately as follows: C57, 26.9 Gm.; C3H, 25.1 Gm.; A, 24.8 Gm.; CBA, 24.9 Gm.; D, 24.5 Gm. This order corresponds in a general way to the order we found in the case of the control mice, although the differences between the different strains are here not very marked and not so great as in the control mice. As to the three groups—males, virgin and breeding female mice—both in the treated and in the control mice, the weights of the males and the breeding females exceed those of the virgin female mice.

DISTRIBUTION OF GRADES OF PROLIFERATION IN THREE WEIGHT CLASSES

So far, groups of mice treated in various ways have been arranged according to the grades which their mammary glands received with respect to proliferation. The average weights which were associated with the different grades were compared and at the same time the ages of these groups of mice were noted. We now wish to analyze our findings in the opposite way, by arranging the mice according to weight classes and then comparing the grades of proliferation of the mammary glands associated with each weight class.

In table 9 the mice of the various strains are classified according to three weight classes: Class I comprises mice weighing 20 Gm. or less;

TABLE 9.—*Distribution of Grades in Three Weight Classes*

Group	Weight Class *	Mice †	Grade
Strain D			
Female controls	I	4	1
	II	10	4
	III	9	4
Ovariectomized controls	I	0	
	II	4	1
	III	1	0.75
Male and female mice given 100 or more rat units of estrogen by injection	I	1	1.75
		4	2.25
		1	4
	II	1	0
		1	1
		1	1.25
		2	2.25
		9	4
	III	1	2
Male and female mice given less than 100 rat units of estrogen by injection	I	6	1
		2	1.25
		3	2.25
		2	4
	II	1	1
		2	1.25
		3	1.75
		1	2.75
		3	3
		12	4
	III	1	1.25
		5	4
Strain C3H			
Female control	I	0	
	II	1	2
		5	4
	III	1	2.25
		6	4
Ovariectomized controls	I	0	
	II	1	1
	III	4	1
Male and female mice given 100 or more rat units of estrogen by injection	I	1	0
		1	1
		2	1.25
		3	1.75
		1	2
		5	2.25
		1	2.75
		1	4
	II	2	0
		2	1
		4	1.25
		2	1.75
		2	3
		15	4
	III	1	0
		5	4
Male and female mice given less than 100 rat units of estrogen by injection	I	1	1.25
	II	3	1
		1	2
		1	2.75
		5	4
	III	2	1
		1	1.25
		1	2.75
		8	4
Strain A			
Female breeding controls	I	0	
	II	6	1
		2	1.25
		1	2
	III	1	0.75
		2	1
		1	2
		2	4

TABLE 9.—*Distribution of Grades in Three Weight Classes—Continued*

Group	Weight Class *	Mice †	Grade
Female virgin controls	I	0	
	II	10	0.75
		13	1
		1	3
		2	4
	III	5	1
		1	4
Ovariectomized controls	I	0	
	II	3	0.75
	III	1	0.75
Male and female mice given 100 or more rat units of estrogen by injection	I	1	0
		3	1
		2	1.25
		2	1.75
		1	2
	II	1	0.75
		27‡	1
		11	1.25
		5	1.75
		3	2.25
		14	4
	III	1	0.75
		1	1
		2	2
		4	4
Male and female mice given less than 100 rat units of estrogen by injection	I	1	0.75
		8	1
		2	1.25
		1	2.75
	II	7	1
		2	1.75
		1	1.75
		1	3
		7	4
	III	1	1
		10	4
Strain New Buffalo			
Female controls	I	1	1
	II	1	1
		1	2
		1	2.25
	III	4	4
Ovariectomized controls	I	0	
	II	8	1
	III	1	0.75
		2	1
Male and female mice given 100 or more rat units of estrogen by injection	I	4	0
		1	1.25
		1	2
		2	2.25
	II	2	0
		1	2.25
		1	4
	III	1	2.75
		2	4
Female mice given less than 100 rat units of estrogen by injection	I	0	
	II	1	2
	III	1	2
		2	2.25
		1	2.75
Strain Old Buffalo			
Female controls	I	0	
	II	1	0.75
		8	1
		2	1.25
	III	4	1
		2	4
Ovariectomized controls	I	1	1
	II	1	0.75
		8	1
	III	7	0.75
		6	1

TABLE 9.—*Distribution of Grades in Three Weight Classes—Continued*

Group	Weight Class *	Mice †	Grade
Male and female mice given 100 or more rat units of estrogen by injection	I	1	2
		1	2.25
	II	1	2
	III	1	2.25
		1	2.75
		4	4
Female mice given less than 100 rat units of estrogen by injection	I	1	1
	II	1	0.75
		5	1
		1	2.25
		1	3.25
	III	0	
Strain CBA			
Female controls	I	2	1
		1	3
	II	6	1
		2	4
	III	0	
Ovariectomized controls	I	0	
	II	2	1
	III	0	
Male and female mice given 100 or more rat units of estrogen by injection	I	1	2
		3	2.25
	II	2	1
		6	1.25
		4	1.75
		4	2
		5	2.25
	III	1	1
		4	1.75
		1	2
Strain C57			
Female controls	I	5	1
	II	22	1
		1	1.25
	III	1	1
Ovariectomized controls	I	3	1
	II	7	0.75
		3	1
	III	6	0.75
Male and female mice given 100 or more rat units of estrogen by injection	I	2	0.75
		4	1
		1	1.25
	II	1	0.75
		13	1
		3	1.25
		4	1.75
		1	2
		2	2.25
	III	1	0.75
		1	1
		4	1.25
		6	1.75
		3	2
		1	2.25
Female mice given less than 100 rat units of estrogen by injection	I	2	1.25
		1	1.25
	II	6	1
		1	2
		1	2.25
	III	1	1.25

* Class I comprises mice weighing 20 Gm. or less; class II, mice weighing between 20 and 30 Gm., and class III, mice weighing above 30 Gm.
† The number signifies the number of mice available.
‡ Fifteen of these mice were ovariectomized.

class II, mice weighing between 20 and 30 Gm., and class III, mice weighing above 30 Gm.

In evaluating the figures given in table 9 it is necessary to consider the following facts: 1. Up to a certain period of life, the weight of mice increases with increasing age. In a general way it may therefore be assumed that the greater the average weight in a certain class, the greater is the average age. However, this relation between weight and age holds good only up to a definite point in the weight class; when this limit has been reached, increasing weight no longer corresponds necessarily to older age.

2. There is a certain correlation between the weight of the mice and the development of their mammary glands, in particular, the development of carcinoma of the mammary gland.

3. One must expect in special cases certain irregularities in the relation between the figures for the average weight and the grades of the mammary gland proliferation, owing to the small number of mice available in a number of classes and to differences in time of death of animals in the various classes which are to be compared. Thus it may happen that the time of death of mice which have received injections of large doses of estrogen may be earlier than that of nontreated animals; this factor may tend to make the grades of the mice given injections of large doses of estrogen relatively too low. In nontreated mice, the highest grades were reached in strains D and C3H in weight class II, and the grades continued high in weight class III. In strain A mice, with a somewhat less strong hereditary tendency to the development of mammary gland carcinoma, the highest grades were reached in weight class III, but even here the grades reached were not very high, and they were lower in virgin than in breeding mice. In strains New Buffalo and Old Buffalo, the highest grades were reached in weight class III. CBA control mice did not happen to reach weight class III, and the highest grades were therefore in weight class II. In strain C57 the grades were low throughout, irrespective of weight. In ovariectomized mice the grades were 1 or less in all strains and in all weight classes, irrespective of the degree of their hereditary tendency to the development of carcinoma of the mammary gland.

Mice Treated with the Largest Doses of Estrogen.—Among mice under the greatest stimulation with estrogen, the highest grades in strains D and C3H were reached in weight class II and the grades in strain C3H continued high in weight class III, but in strain D only 1 mouse belonged to weight class III. In strain A mice, the greatest effect began to be reached in weight class II, but the highest grade was attained in weight class III, although even here the average grade reached was not very high, in accordance with the fact that in our laboratory

strain A mice had a decidedly lower hereditary tendency to the development of mammary carcinoma than strains D and C3H. In strains New Buffalo and Old Buffalo, given large doses of estrogen, the highest grades were reached in weight class III, at a time when the mice had attained a greater weight than mice possessing a greater hereditary tendency to the development of mammary carcinoma. In strain CBA, the grades were as high in weight class I as in weight classes II and III, but it seemed that injection of estrogen increased the number of intermediate grades over that found in the nontreated animals. In strain C57, injection of the largest doses of estrogen raised the grades only to a very moderate degree; the relatively highest grades were in weight class III, although even here the grades were relatively not very high, in accordance with the low degree of hereditary tendency to the development of mammary carcinoma which is characteristic of this strain.

Mice Given Smaller Doses of Estrogen.—Among mice treated with smaller amounts of estrogen, the highest grades in strains D and C3H were reached, or almost reached, in weight class II, and the high grades continued in weight class III, but in some instances the highest grades were attained only in weight class III. In general, the preponderance of weight class II was here perhaps not quite so great as in mice treated with the larger doses of estrogen. In strain A mice the maximum effect was reached in weight class III, in which the results obtained were much superior to those in weight class II. On the whole, therefore, the preponderance of weight class III over weight class II in these three strains was more definite in mice given smaller rather than larger doses of estrogen and in those with less than the highest degree of hereditary tendency to mammary carcinoma. In strain New Buffalo, the greatest effects were in weight class III. In strain Old Buffalo, no mice were available in class III. In both of these strains the average grades in the higher weight classes were less high than those obtained in mice treated with the largest doses of estrogen. In strain C57 the grades in weight class II were higher than those in weight class I, while in weight class III the number of mice available was too small for comparison. The grades in all the mice belonging to this strain remained relatively low; furthermore, here improvement of the grades with increase in weight was less marked than in the mice given larger doses of estrogen.

COMMENT

In stating the main conclusions which may be drawn from these statistical data, it will be necessary at the same time to consider the difficulties encountered in the evaluation of the data. For a correct comparison of the weight of tumor mice with that of nontumor mice, one must first deduct from the former the weight of the tumor in order to

obtain the net weight of the animals. In the treated mice, the tumors were usually small at the end of the experiment, when the final weight was taken. In the case of the control mice, some of the tumors were large, but making full allowance for this factor, the difference in weight caused by the presence of a tumor is not sufficient to explain the difference in weight found between tumor-bearing mice and mice without tumors. From our data the conclusion may therefore be drawn that a greater weight of the mice is one of the factors which favors the development of mammary carcinoma. A greater weight of the tumor mice is found in males as well as in breeding and virgin females; it is also found in the various strains in which the number of tumors which develop is large enough for statistical purposes. The effect of weight on the development of mammary carcinoma is less clear in a few smaller subdivisions, such as castrated males which received ovarian transplants and some mice with anterior hypophysial transplants, but in these groups the number of mice used was relatively small.

In normal mice, the weight is greatest in males and in breeding female mice, and it is lowest in virgin mice. It reaches a maximum in these groups at the age of from 9 to 11 months. Subsequently, no marked increase seems to take place, although some variations may occur. There is a possibility that in mice older than 20 months the weight reaches a new maximum, although it is not certain that in this case we may not have to deal with accidental variations. Strong indications exist that in different strains the average weights differ, and it is of interest in this connection that although a higher weight of mice favors the development of tumors, it is not the strains with high tumor rates (D, C3H and A) which have the highest weight averages but, on the contrary, some strains with a relatively low hereditary tendency to the development of mammary gland carcinoma, such as the Buffalo and C57 strains. The weight of mice is therefore influenced by various factors. The strain differences which exist are evidently of a hereditary nature. The difference in the tendency to the development of mammary carcinoma in various strains depends on inherited factors, which are so strong that they greatly overbalance the effects of differences in weight. On the other hand, there is a parallelism between the growth and aging curves of the skeletal system (M. and R. Silberberg [2]) and the incidence of carcinoma of the mammary gland in these various strains. But, in addition, there are factors active within the various strains which help to determine which individuals belonging to a certain strain shall be affected by mammary carcinoma, and here the weight of the individual mice plays a certain role. In mice treated with estrogen, the average weights at different ages and in the different strains are very similar to those in

2. Silberberg, M., and Silberberg, R.: Am. J. Anat. **68**:69, 1941.

the control mice. In the latter the maximum is reached between the age of 9 and 20 months. We had expected to find lower average weights in the mice treated with estrogen, but this was actually not the case. Moreover, the order in which the weight changes in the different strains is similar in the control and the treated mice.

In the evaluation of the significance of weight for the growth of the mammary gland and carcinoma of the mammary gland we face the difficulty that our records usually do not indicate whether a low weight had been acquired toward the end of the experiment, perhaps as a result of accidental factors, such as intercurrent chronic disease, or whether it had been present during a great part of the experiment; in the latter case, the low weight should have had a better chance to affect the growth of the mammary gland and the development of a tumor than in the former case. Although a loss of weight which occurred only a short time before the autopsy may have been present in a number of cases, these cases were probably not of sufficient frequency to outweigh the effect of a change in weight which extended over a longer period.

If the data recorded here prove that the weight of an individual enters as a factor into the development of mammary carcinoma, the question remains as to the phase in the process of cancerization which is affected by changes in weight. Do such changes affect the growth processes in the mammary gland which precede the development of carcinoma or do they affect only the last phase of this growth, the transformation of the normal into the precancerous and cancerous growth, or do they affect all of these processes? It has not been possible to prove by the statistical means used by us that there is a definite relation between the weights of an animal and the intensity of the normal growth processes in the mammary gland. But our data are perhaps not suited for the demonstration of such a relation, because the grades given by us to the mammary gland disregard the extensiveness of the growth and merely express the intensity of the growth, irrespective of whether it is limited to small areas of a gland or extends over wider portions. If the extensiveness of growth had also been considered in attaching grades to the various growth stages, a relation between this condition and the weights of the individual mice would perhaps have become manifest. In fact, by microscopic study of the mammary glands it has been possible to show that deficiencies in nutrition as expressed in low weight cause extensive changes in the fat tissue of the mammary gland, which are followed by destruction of the ducts and acini of the mammary gland and which thus diminish the chances for the development of carcinoma. Furthermore, in cases of undernourishment we often find a considerable absolute or relative increase in the amount of hyaline tissue surrounding the ducts and also the acini of the mammary gland, and this hyaline coat likewise

may lead to degenerative changes in the structures of the mammary gland. It is also possible that in some instances other factors have complicated and covered up such a relationship. But, notwithstanding these difficulties, in the C57 strain there is an indication that increased weight of the mice was associated with increased noncancerous growth of the mammary gland. The assumption that the weight of an individual is of some significance in the preparatory growth processes of the mammary gland is probable also because in various organs it has been shown that a relation exists between cell multiplication and the state of nutrition.

If, instead of classifying the mice according to age, we classify them in weight classes, we find that the grades representing the growth of the mammary gland, including its last stage, mammary carcinoma, increase with increasing weight. Further, as in the various age classes, we find in the weight classes that mice belonging to strains with a greater hereditary tendency to mammary carcinoma show the higher grades of mammary growth at a somewhat lower weight than do strains with a lower hereditary tendency. Similarly, after injection of larger doses of estrogen the peak of mammary growth is reached in a lower weight class than after injection of smaller doses of estrogen.

We see, then, a remarkable parallelism between the significance of weight and that of age for the growth of mammary adenocarcinoma in mice. There is noticeable, in the tables in which the animals are arranged in weight classes, not only an increase in the incidence of mammary carcinoma with increase in weight but also a rise in the averages of the intermediate grades, indicating intensification of the growth processes in the mammary gland. This is understandable because the weight of the individual animals is determined to a large degree by their age; but it is possible, and it may even seem probable, that, in addition, increasing weight as such improves the growth of the mammary gland before the point of cancerous growth has been reached. But even in view of the lack of concordance between the intermediate grades of the mammary glands and the average weights of the animals in tables 5 and 6, where the arrangement is according to grades given to the mammary glands, it still would not be necessary to conclude that weight influences only the specific process of cancerous transformation and not the preceding growth phase.

There remains the question as to whether the effects on cancerous growth are due to the loss in weight of the body as a whole, and of the organs and tissues composing the organism, caused by the deficient use of various kinds of important foodstuffs, or whether they are due to a deficiency in a specific growth substance associated with the general loss in weight and running parallel to the latter. Both of these possibilities exist, but at present it does not seem possible to decide between them.

To recapitulate the main conclusions: This statistical study of normal control mice and of mice treated with various glandular substances shows that the mice which are bearers of mammary tumors have a greater weight than mice not having tumors and, conversely, that mice that have a greater weight have a greater tendency to acquire mammary tumors than mice with a lower weight. While we could not prove directly that the growth processes preceding and leading to the formation of mammary carcinoma are likewise associated with greater weight, there are certain observations concerning the effect of underfeeding on the mammary glands of mice, as well as other facts concerning the general effects of undernourishment on tissue growth, which favor this conclusion. It is possible that in the system of grading used in these investigations the relation between weight and mammary growth becomes manifest only when the later stages of these growth processes, consisting in the beginning period of cancerous growth, have been reached. It has further been shown that relations exist between the weight curves of mice, on the one hand, and the hereditary tendency to the development of mammary carcinoma, as well as the doses of estrogen administered, on the other, and these relations are similar to those which we have formerly observed in studying the age factor in the origin of tumors.[1c]

It was observed a long time ago that the state of nourishment influences the growth of tumors, but these early observations concerned the behavior of transplanted tumors rather than the origin and development of spontaneous tumors. Moreschi,[3] Sugiura and Benedict,[4] Bischoff, Long and Maxwell[5] and others noted a retardation of the growth of transplanted tumors as a result of underfeeding. Rous[6] and subsequently Sugiura and Benedict[4] found that long-continued underfeeding delayed the recurrence of incompletely extirpated or transplanted spontaneous tumors. But the investigations reported here are more similar to the more recent extensive experiments of Tannenbaum,[7] who found that underfeeding caused retardation in the appearance and diminution in the number of epithelial and connective tissue tumors which otherwise would have developed as the result of the application of 3, 4-benzpyrene. Similar observations were made with spontaneous mammary and pulmonary tumors in mice. Also, in the case of human cancers the

3. Moreschi, C.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **2**:651, 1909.

4. Sugiura, K., and Benedict, S. R.: J. Cancer Research **10**:309, 1926.

5. Bischoff, F.; Long, M. L., and Maxwell, L. C.: Am. J. Cancer **24**:549, 1935. Bischoff, E., and Long, M. L.: ibid. **32**:418, 1938.

6. Rous, P.: J. Exper. Med. **20**:433, 1914; Bull. Johns Hopkins Hosp. **26**:146, 1915.

7. Tannenbaum, A.: Am. J. Cancer **38**:335, 1940; Arch. Path. **30**:509, 1940.

development of the tumors seems to take place preferably in well nourished persons, as Tannenbaum [7] concluded from the study of life insurance statistics.

SUMMARY

In normal (control) mice there is a weight curve which reaches a peak between the ages of 9 and 11 months and then may show certain variations which are probably accidental; there is, however, a possibility that in mice older than 20 months the weight curve may rise to a still higher point. The weight curves, as well as the average weights at different ages, of mice given injections of estrogen were similar to those of nontreated control mice.

Different strains of mice differ in average weights at various ages, and their weight curves are not parallel to the hereditary tendency of these strains to acquire mammary carcinoma. The differences in weight curves in various strains of mice are similar to those in normal animals and in animals treated with estrogen.

Within the various strains possessing a sufficient tendency to the development of mammary carcinoma, there exists a direct relation between the frequency of mammary carcinoma and the average weight, the latter being greater in tumor-bearing mice.

Although it has not been possible to prove statistically that in non-cancerous mice the growth intensity of the mammary gland shows the same relation to the weight curve as does the carcinomatous end stage, certain direct observations as well as more general considerations make it probable that the state of nutrition influences the development of mammary carcinoma largely by influencing the preparatory growth processes in the mammary gland. Such a relation becomes manifest also if we use the weights of the mice rather than the grades of mammary gland proliferation as the principle of classification.

The greater the hereditary tendency to the development of mammary carcinoma in various strains of mice, or the larger the doses of estrogen given, the more readily was the cancerous stage reached in mice belonging to a lower weight class; this is in agreement with our previous findings, in which a similar relation was established between the frequency of mammary carcinoma and the age class to which the animals belonged.

OSTEOCHONDRITIS DEFORMANS OF THE HIP (LEGG–PERTHES DISEASE) AND RENAL OSTEITIS FIBROSA CYSTICA

REPORT OF A CASE WITH ANATOMIC STUDIES

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The patient whose case is reported in this communication suffered from bilateral necrosis of the femoral capital epiphyses (Legg-Perthes disease) and from diffuse osteoporosis of the skeleton, attributable to severe chronic renal insufficiency (renal osteitis fibrosa cystica).[1] The chief purpose of the report is to place on record a detailed description of the anatomic changes observed post mortem in one diseased hip joint. The material was made available by the courtesy of Dr. Soma Weiss.

REPORT OF CASE

A 13 year old white boy entered the Peter Bent Brigham Hospital in September 1939, complaining of puffiness of the face, weakness, soreness of the mouth, nausea and vomiting.

The patient's mother suffered from Addison's disease during her pregnancy and died immediately after delivery of the child by cesarian section.

The patient presented a difficult feeding problem in infancy and was always considered to be underweight and underdeveloped.

Two years before admission it was noted that he limped. A diagnosis of bilateral Legg-Perthes disease was established on subsequent examination. About eighteen months prior to admission, polydipsia, polyuria and occasional puffiness about the eyes were noted. One month before admission, the edema of the face increased, and the patient became lethargic. For one week there had been soreness of the mouth, nausea and vomiting, fever, dyspnea, oliguria and epigastric pain.

The patient's appearance was that of a child of about 7 years of age. There was symmetric puffiness about the eyes and cheeks, and the skin appeared waxy and pale yellow. The breath had an ammoniacal odor. The mucous membranes

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From the Department of Pathology, Harvard Medical School.

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1. Albright, F.: Tr. A. Am. Physicians **51**:199, 1936. Albright, F.; Drake, T. G., and Sulkovitch, H. W.: Bull. Johns Hopkins Hosp. **60**:377, 1937.

of the oral cavity were covered by a heavy layer of whitish material, and small bleeding points were noted along the gingival margins. The heart was moderately enlarged. There were a diffuse precordial systolic thrill and a grade 3 systolic murmur at the apex. The systolic blood pressure was 150 mm. of mercury; the diastolic, 80. The lungs were not abnormal to percussion and auscultation. The abdomen and the extremities revealed no abnormalities. No nodules were palpated in the region of the thyroid gland.

Laboratory Data.—The red blood cell count was 960,000. The hemoglobin content was 15 per cent. The white blood cells numbered 11,600, and the differential leukocyte count showed 87 per cent neutrophils and 13 per cent lymphocytes. The blood smear was otherwise normal. The urine had a specific gravity of 1.010. It contained a trace of albumin, occasional red blood corpuscles, a few leukocytes and cellular casts. The icteric index was 3. The Wassermann and Hinton tests of the blood were negative. The phenolsulfonphthalein excretion was 0 in two hours (90 cc. of urine).

Chemical studies of the blood serum gave the following results:

	In 100 cc.
Nonprotein nitrogen	375 mg.
Urea nitrogen	200 mg.
Serum protein	6.1 Gm.
Albumin	2.7 Gm.
Globulin	3.4 Gm.
Sodium chloride	540 mg.
Calcium	5.9 mg.
Phosphorus	17.4 mg.
Carbon dioxide-combining power	16 vol. %

Interpretation of the Roentgenograms.—The heart was described as markedly enlarged and roughly triangular. The lungs contained areas of consolidation that suggested bronchopneumonia. The long bones showed increased radiolucence, and there was evidence of delayed development of the epiphyses of the lower end of the radius and ulnar styloid process (fig. 1 *A*). The femoral heads showed the characteristic deformities of bilateral Legg-Perthes disease, more advanced on the right side (fig. 1 *B*). There were six lumbar vertebrae, with sacralization of the right transverse process of the sixth.

Course.—On the day following admission of the patient to the hospital, moist rales developed in both lungs, the patient became progressively more dyspneic, lapsed into coma and died.

Necropsy.—The general appearance of the body was that of a child considerably less than the stated age of 13 years. The face was puffy, the skin pallid, and there was considerable edema of the scrotum. The serous cavities contained a slight excess of clear fluid. The thyroid gland showed inclusion of a pyramidal lobe. Four structures believed to be slightly enlarged parathyroids were removed for microscopic examination. The heart weighed 200 Gm. The following minor congenital anomalies were observed: The pulmonary vein terminated in the coronary sinus; the left circumflex coronary artery was anomalous in distribution, there were multiple ostiums for the right coronary artery, and there was slight narrowing of the mitral ring without abnormality of the cusps. The lungs contained several small areas of consolidation consistent with bronchopneumonia.

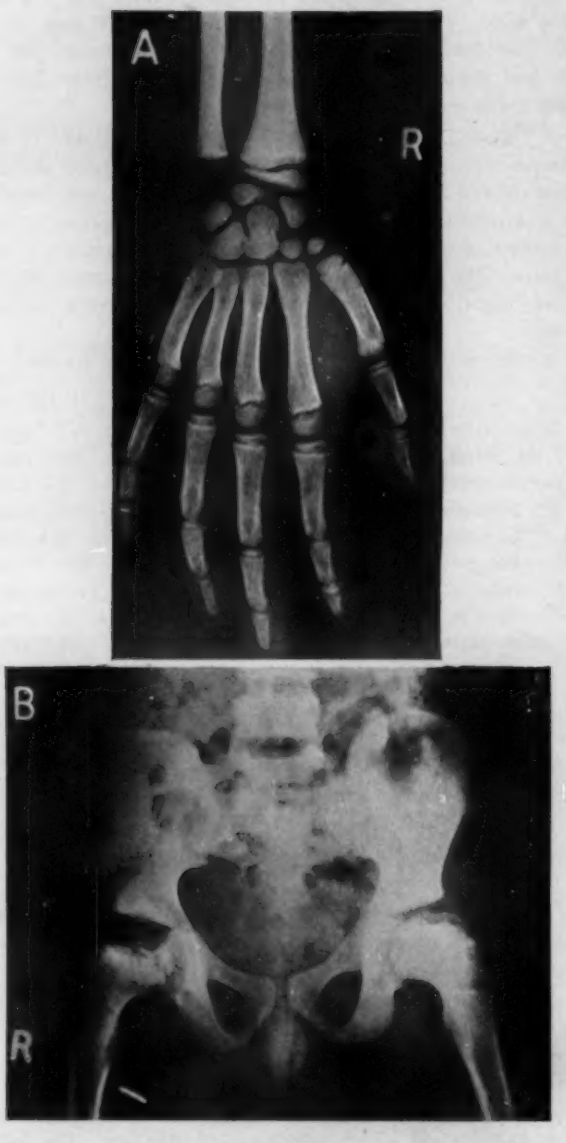

Fig. 1.—*A*, roentgenogram of the right hand. The bones show increased radiolucence and delayed development of the radial and ulnar styloid epiphyses. The appearance of the epiphyses in general is not that of true rickets.

B, roentgenogram of the pelvis. The displacement and deformity of the femoral heads are characteristic of Legg-Perthes disease. Sacralization of the right transverse process of a sixth lumbar vertebra is also shown.

The right kidney and ureter were absent, as were the right renal artery and vein. The left kidney weighed 19.5 Gm. (normal, 70 to 95 Gm.[2]), and its capsule was somewhat adherent. The exposed cortical surface was coarsely granular, and a coronal section of the kidney revealed a mottled yellowish pink surface. It was not possible to distinguish the corticomedullary junction except at the polar extremes. There was an anomalous artery to the upper pole. The renal pelvis was not obviously dilated but appeared larger than the amount of kidney substance present might require. Its mucosa was pale yellowish white. The ureter was moderately dilated, but its caliber was reduced in the intravesical portion. The bladder and the prostate were normal.

The other organs revealed only minor congenital abnormalities. The gallbladder was suspended by a mesentery. There was an exaggerated cleft in the spleen, resulting in a bifid appearance, with the lower portion projecting into the lesser peritoneal sac. An annular process of the pancreatic tissue extended from the head to encircle the second portion of the duodenum. A small traction diverticulum was present on the medial aspect of the second portion of the duodenum. In the right lateral paracolic region near the origin of the right leaf of the diaphragm was a 1.0 cm. grayish white peritoneal cyst.

Right Hip Joint: Gross examination of the resected hip showed the femoral head to be markedly flattened on its superior surface but covered by smooth normal-appearing articular cartilage. The acetabulum appeared normal. The synovial tissues showed slight villous hypertrophy and congestion. The ligamentum teres was flattened and lengthened (2 cm.), and its course was more oblique than normal.

A roentgenogram of the excised specimen showed considerable rarefaction of the epiphysis of the femoral head with an area of decreased density extending back into the neck for a distance of about 2.0 cm. The absence of condensation in the epiphysis and the marked decalcification of the neck were features which the roentgenologist (Dr. M. C. Sosman) did not consider characteristic of Legg-Perthes disease.

Subsequent to decalcification in 5 per cent aqueous nitric acid solution, the femoral head and neck and the acetabulum were sectioned longitudinally. Examination of the cut surfaces revealed a markedly shortened and widened femoral neck (fig. 2). The relative increase in width was greatest (4 cm.) near the femoral head. The neck measured only 3 cm. in length. Flattening of the femoral head was most marked in an area lateral to and above the insertion of the ligamentum teres. The smaller mesial and inferior portion of the articular surface was rounded and had a configuration that approximated the normal. The epiphysial cartilage of the upper femoral epiphysis was narrow and very irregular in outline. In places it could not be recognized. The bony tissue in the femoral head was compressed and near its center was yellowish and appeared fragmented. The greatest change had occurred in that portion of the epiphysis which lay lateral to the insertion of the ligamentum teres.

Microscopic Examination.—(*a*) Kidney: All portions of the kidney were severely altered. The capsule was scarred and adherent to the renal cortex. Glomeruli were few. Of those remaining, some were cellular and bloodless, while others were fibrosed or hyalinized. The epithelial cells lining the capsular spaces were prominent, but no "crescents" were seen. There was diminution in the number of tubules. The convoluted portions were arranged in adenoma-like islands

2. Coppoletta, J. M., and Wolbach, S. B.: Am. J. Path. **9**:55, 1933.

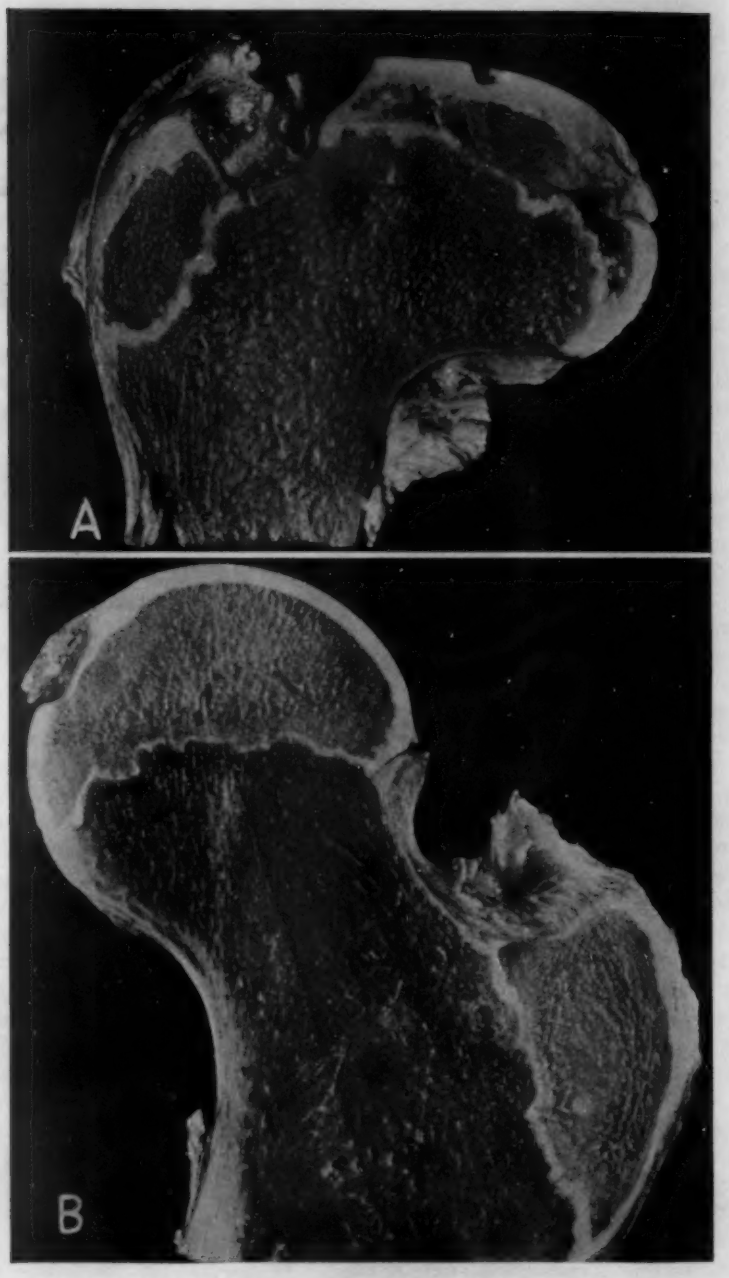

Fig. 2.—*A,* longitudinal section through the upper end of the right femur in the present case of Legg-Perthes disease. The collapse, displacement and attendant deformity of the femoral head are well shown. The comparative preservation of articular cartilage is also apparent.

B, longitudinal section through the normal upper end of a femur in a boy 13 years of age. The illustration is included for contrast with the specimen shown in *A.*

and contained widely dilated lumens filled with a granular basophilic precipitate. The epithelial cells were flattened and exhibited varying degrees of degeneration. Similar degenerative change appeared within collecting tubules, and there was considerable epithelial desquamation as well. These channels were only moderately dilated but contained a wide variety of material. In a few there were small clusters of red cells, in others clumps of polymorphonuclears, but in the majority there were masses of a homogeneous and eosinophilic colloid-like substance. The interstitial substance was increased in amount and densely fibrous. Abundantly scattered throughout were large numbers of lymphocytes, often forming loosely arranged clusters. There were, in addition, a few interspersed plasma cells, monocytes and rare polymorphonuclears. The arterial walls were moderately thickened as a result of muscular hypertrophy. In a few of the larger arteries subintimal fibrosis was noted. Although the mucosa of the renal pelvis was intact, the submucosal layers were fibrotic and markedly infiltrated by lymphocytes.

(*b*) Parathyroids: Sections of two glands were studied. One of these glands was embedded beneath the thyroid capsule. Both parathyroids were comprised of closely packed cells of the "chief" and "transitional water clear" types. There was pronounced nuclear uniformity, and no mitotic figures were seen. Interspersed were small numbers of "oxyphil" cells, some of which formed groups consisting of six to eight cells. One gland contained minimal amounts of fat.

(*c*) Skeletal System: The femoral neck and head revealed the deformities described in the gross (fig. 3 *A*). The superior border of the markedly shortened neck was capped and overhung by the flattened articular cartilage surface so that on the superior surface the femoral neck was but 0.6 cm. in length. In proportion to its length, the femoral neck was greatly increased in its vertical diameter. The articular cartilage of the malformed head was smooth save for an abrupt depression (1.5 mm. deep) which marked the insertion of the ligamentum teres (fig. 3 *A*). Medial to this point the bony structure of the capital epiphysis varied little from the normal and the articular cartilage was normal in thickness. The calcified layer of cartilage and the adjacent subchondral bone showed only the resorptive changes that were common to the other bone tissues examined. Over the lateral two thirds of the femoral head the articular cartilage was thickened and stained lightly with eosin dye. In the deeper portion of the cartilage, the matrix was fibrillated, and the cells were clustered together or imperfectly alined. Nearly all of the calcified layer of cartilage with its subjacent bone trabeculae had disappeared. As a result the articular cartilage was joined with the fibrous tissue replacing the bony epiphysis. One fragment of necrotic subchondral bone of only a few millimeters' size remained in place (fig. 3 *B*). Deeper in the altered femoral head there were spicules of bone and unidentified calcareous material, and in one region a large multiloculated area of cystic degeneration had developed (fig. 4 *A*). Adjacent to the latter were numerous acidophilic strands of noncellular fibrin or fibrin-like material. There were also moderate numbers of mononuclear phagocytic cells. Many of these contained hemosiderin. In several regions the fibrous tissue which had replaced the bony epiphysis was well vascularized. A wide band of highly vascular connective tissue penetrated deeply into the necrotic mass through a disrupted segment of the epiphysial cartilage plate from the neck of the femur (fig. 4 *B*).

The epiphysial cartilage plate was very irregular in contour and thickness. In the area directly beneath the necrotic portion of the femoral head, it was entirely missing, and the cancellous bone of the neck merged with fibrous tissue which had replaced the bone of the epiphysis. The medial third of the epiphysial

cartilage layer was much better preserved. It was in this area only that evidence of the normal sequences of endochondral ossification could be seen. Even in this better preserved portion the cartilage cell columns were markedly deranged and the primary bone trabeculae imperfectly formed. Similar abnormalities were

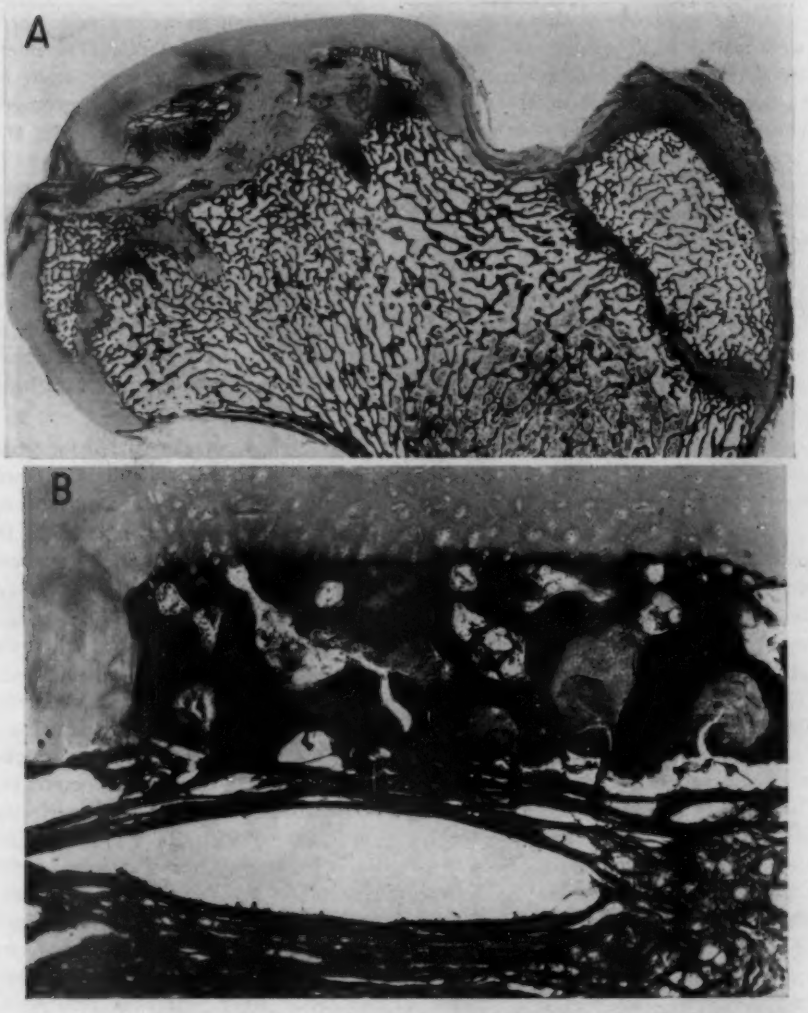

Fig. 3.—*A*, photograph of a section of the right femur; × 2. There is marked flattening of the intact articular cartilage as the result of the collapse of the bony structure of the head. The trabecular structure of the head is destroyed except at a point medial to the insertion of the ligamentum teres. Here, save for compression, the structure is preserved.

B, small area of partially preserved but nonviable subchondral bone; × 39.5. The trabeculae, although retaining natural outlines, are dark staining, and their lacunae are devoid of osteocytes. The articular cartilage, except for minor degenerative changes, retains its normal appearance. Marrow subjacent to the calcified cartilage layer is necrotic and contains a flattened cystlike crevice.

noted in the epiphysial cartilage of the great trochanter. It was apparent that bone growth was markedly retarded throughout the upper femoral epiphysis. In addition there had been complete cessation of the normal growth sequences over the outer two thirds of the femoral neck.

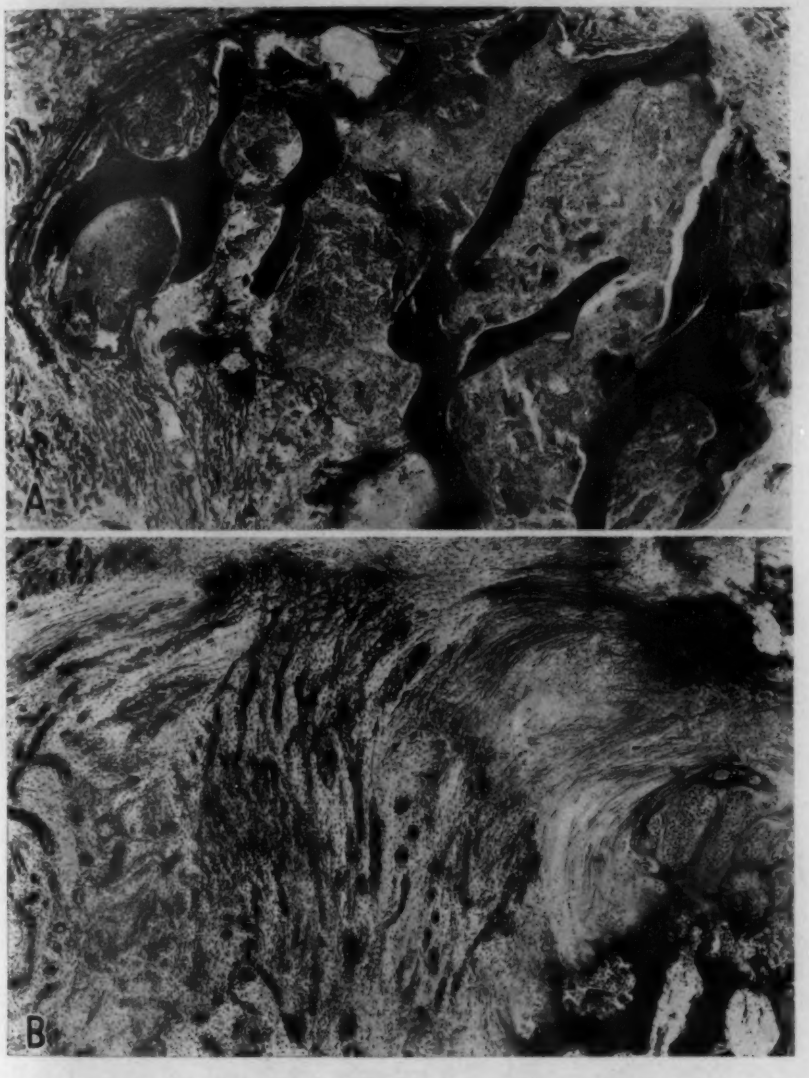

Fig. 4.—*A*, island of necrotic bone and marrow tissue in the femoral head; × 36.5. Evidence of beginning organization is apparent at the right upper margin of the photograph.

B, strand of vascular connective tissue which extended through a defect in the degenerated epiphysial cartilage to enter the necrotic head is illustrated in this photomicrograph; × 36.5.

Small blood vessels entered the margins of the femoral head from the periosteal and perichondrial surfaces. These vessels, as well as numerous arteries and veins seen in the ligamentum teres, showed no evidence of any type of occlusion.

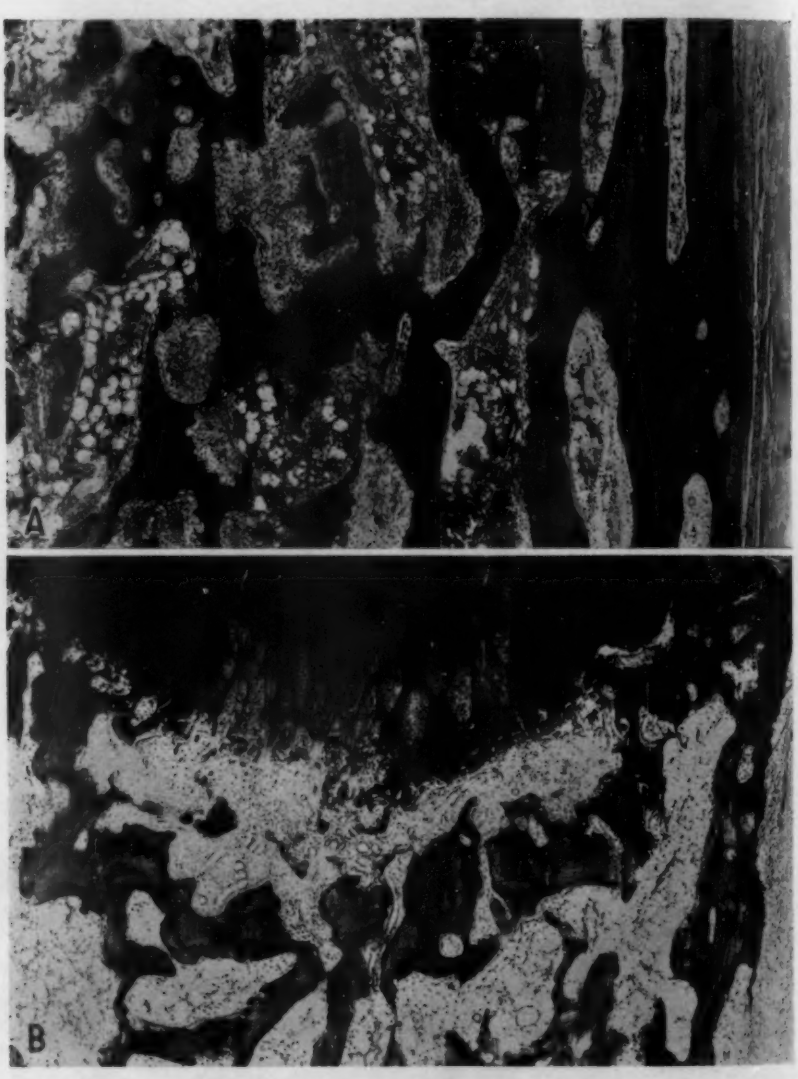

Fig. 5.—*A*, section of rib showing marked resorptive changes consistent with osteitis fibrosa cystica; × 36.5. The trabeculae are thinned and irregular as the result of osteoclasis. Each trabecula is ensheathed by a layer of moderately cellular connective tissue encroaching to a moderate degree on otherwise normal marrow.

B, section at a costochondral junction; × 39. The chondrocytic growth columns are widened, chondrocytes are clustered, and primary calcified trabeculae are minimal in number. Growth was apparently either markedly impeded or completely interrupted. Irregular trabeculation and fibrosis are present in the adjacent marrow.

Sections of the acetabulum showed increased lacunar resorption of bone and irregularities in endochondral ossification. The attached synovial membrane appeared normal.

Microscopic examination of several ribs, including their costochondral junctions, revealed pronounced increase in lacunar resorption of bone (fig. 5 *A*) and marked disturbance in endochondral ossification. The thinned and uneven bone trabeculae were ensheathed in strands of cellular fibrous tissue, which appeared to occupy chiefly the areas formerly comprised of bone. It was apparent, however, that there had been some fibrous tissue overgrowth with encroachment on the marrow spaces. Bone resorption, although present throughout the rib shaft, was more pronounced in a zone 1.5 cm. in width adjacent to the costochondral junction. The cancellous portions were more markedly affected than the cortex. Although the predominant bone change was one of resorption, there was a considerable degree of bone-forming activity. This was manifested by rows of osteoblasts alined along portions of the residual bone trabeculae. Beneath such rows of osteoblasts were seams of newly deposited osteoid matrix of variable thickness. The marrow tissue intervening between the bony and the fibrous trabeculae was in part fatty and in part hemopoietic.

The zone of endochondral ossification in the ribs was comprised of irregularly arranged and markedly widened columns of cartilage cells. Large rounded or oval-shaped cell aggregates were present in the deepest zone (fig. 5 *B*). The intervening cartilage matrix showed marked variations in its staining reaction, some portions staining very lightly, while other portions at the same level stained intensely with hematoxylin. There was marked diminution in the number of primary cartilaginous trabeculae. This was particularly marked near the center of the shaft, where the subchondral bone was almost entirely separated from the cartilage by an intervening layer of cellular and vascular connective tissue. The better formed cartilage trabeculae were not surrounded by the normal amount of osteoid tissue. Blood vessels and capillary channels at the bone ends were irregularly arranged, and there was little evidence of capillary penetration into the cartilage cell columns. The observed microscopic changes indicated marked retardation in growth sequences. It appeared probable that this disturbance had resulted from the pronounced and active bone resorption rather than from any specific vitamin deficiency.

Autopsy Diagnosis.—Chronic pyelonephritis; agenesis of the right kidney and ureter; secondary parathyroid hyperplasia; generalized osteitis fibrosa cystica (renal); bilateral Legg-Perthes disease; terminal Staphylococcus aureus septicemia; bronchopneumonia.

In 1910 Legg,[3] Perthes [4] and Calvé [5] published independent observations on the type of hip joint disease that serves as the basis for this report. Although the lesion has since received wide attention from orthopedic surgeons and roentgenologists, the literature contains little specific information concerning the causes or the pathogenesis and relatively few detailed pathologic descriptions.

3. Legg, A. T.: Boston M. & S. J. **162**:202, 1910.
4. Perthes, G.: Deutsche Ztschr. f. Chir. **107**:111, 1910.
5. Calvé, J.: Rev. de chir. **42**:54, 1910.

Numerous etiologic theories have been advanced.[6] Infection,[7] rickets,[5] various forms of trauma,[8] imbalance of the sympathetic nervous system,[9] congenital deformity of the acetabulum with resultant femoral adaptation,[10] and embolism [11] have been suggested as causative factors. The majority of the authors offering these factors have, however, accepted as the underlying cause an interruption of the normal vascular supply to the capital epiphysis.

The lesion appears with greatest frequency between the ages of 5 and 12 years,[12] a period during which the capital epiphysis is believed to be especially vulnerable to interference with its vascular supply. The circulatory capacity of the ligamentum teres is said to diminish after the age of 2 years and to reach a minimum at the age of 5 years.[8d] During the succeeding period of growth the capital epiphysial cartilage remaining active and unfused serves as a barrier to the rich vascular bed of the marrow cavity of the neck. The major source of nutrient supply consists, therefore, of small vessels which arise near the insertion of the capsule, are reflected subperiosteally along the neck of the femur and enter the head somewhere in the vicinity of the margin of the articular cartilage.[13] It has been suggested that the fineness of these vessels and their location cause them to be particularly susceptible to trauma.[8d] The experimental observations of Cordes,[8a] Kistler,[11b] Nagura and Kosuge [14] and Miltner and Hu [10b] have demonstrated the development of capital necrosis as the result of interference with the circulation in these channels.

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6. Lang, F. J.: Virchows Arch. f. path. Anat. **239**:76, 1922.

7. Kidner, F. C.: Am. J. Orthop. Surg. **14**:339, 1916. Phemister, D. B.: Arch. Surg. **2**:221, 1921. Roberts, P. W.: J. A. M. A. **69**:1598, 1917.

8. (*a*) Cordes, E.: Beitr. z. klin. Chir. **149**:248, 1930. (*b*) Hirsch, E. F.: Arch. Surg. **37**:926, 1938. (*c*) Konjetzny, G. E.: Acta chir. Scandinav. **74**:361, 1934. (*d*) Legg, A. T.: Surg., Gynec. & Obst. **22**:307, 1916. (*e*) Perthes, G., and Welsch, G.: Beitr. z. klin. Chir. **127**:477, 1922.

9. Leriche, R., and Policard, A.: Normal and Pathological Physiology of Bones, St. Louis, C. V. Mosby Company, 1928, p. 214.

10. (*a*) Jansen, M.: J. Bone & Joint Surg. **21**:265, 1923. (*b*) Miltner, L. J., and Hu, C. H.: Arch. Surg. **27**:645, 1933.

11. (*a*) Axhausen, G., and Bergmann, E.: Ernährungsunterbrechungen am Knochen, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1937, vol. 9, pt. 3, chap. 3, p. 167. (*b*) Kistler, G. H.: Arch. Surg. **33**:225, 1936.

12. Legg, A. T.: Am. J. Orthop. Surg. **16**:448, 1918. Sutro, C. J., and Pomeranz, M. M.: Arch. Surg. **34**:360, 1937.

13. Lexer, E.: Arch. f. klin. Chir. **71**:1, 1903. Scudder, C. L.: The Treatment of Fractures, ed. 11, Philadelphia, W. B. Saunders Company, 1938, chap. 36, p. 818. Miltner and Hu.[10b]

14. Nagura, S., and Kosuge, S.: Arch. f. klin. Chir. **191**:347, 1938.

Most of the recent authors have agreed with Zemansky,[15] who contended that mild trauma to the hip injures the subperiosteal vessels directly or by causing slight displacement of the epiphysis. Such injury occurring at an age when the vascular supply through the ligamentum teres had been physiologically diminished would lead to infarction of the bony and medullary substance of the capital epiphysis. The predisposition to necrosis might be expected to be enhanced, according to Nagura and Kosuge,[14] if, as the result of few or no subjective symptoms, the affected person failed to immobilize the limb or to avoid weight bearing.

A loss of vascular supply to the femoral head, although causing necrosis of bone and medullary supportive tissues, has little or no effect on the articular cartilage, which is nourished in part at least by synovial fluid. According to Zemansky,[15] the lesion must show massive necrosis of subchondral bone and marrow, but the viability of overlying articular cartilage should be preserved. The deformity attendant on the collapse of the bony structure naturally subjects the cartilage to unusual stress. As a result, secondary degenerative changes of variable degree in the joint may be anticipated at a later period.

Although it was not possible in the present case to prove that the lesion of the hip joint had resulted from interference with the blood supply to the capital epiphysis, the findings were interpreted as being consistent with this mode of onset. The preservation of trabecular viability in a small area on the medial aspect of the right femoral head was believed to have been possible because of the proximity of this region to the point of entrance of a small perforating artery. Only minor reparative changes were detected in the necrotic trabeculae, and these evidences of repair were associated in part with focal disruption of the epiphysial cartilage where extension of blood vessels from the neck into the femoral head had occurred. This was similar to a focal lesion described by Lippmann.[16] It was not possible to determine whether or not this defect in the cartilage had been caused by an original injury or by a fracture resultant from the deformity of the head. In any case it was apparent that the initial repair process had taken place through revascularization of the femoral head by penetration of blood vessels across the epiphysial plate. Ordinarily the necrotic bone substance is believed to persist, with little evidence of repair, until disruption of the epiphysial cartilage under the stimulus of physiologic fusion has occurred. This permits the entrance of vascular channels from the shaft marrow, and repair and reossification follow. Deformity may be expected to remain as a permanent stigma.

15. Zemansky, A. P.: Am. J. Surg. **4**:169, 1928.
16. Lippmann, R. K.: Am. J. Surg. **6**:785, 1929.

It is generally believed that the lesion of Legg-Perthes disease is histologically and etiologically identical with the aseptic bone necrosis encountered in the navicular bone (Köhler's bone disease) and the tibial tubercle (Osgood-Schlatter disease) and other similar conditions. The observations recorded in the literature and those made in our own case are entirely in keeping with this concept.

There is little reason to believe that there was any relation between the deforming lesion of the hip and the osteitis fibrosa cystica from which this patient suffered. It is probable, however, that the metabolic disturbances responsible for the rarefied state of the skeletal system operated to prevent the development of increased bone density of the femoral neck.

The mechanism of the production of skeletal rarefaction in association with severe and long-standing renal disease such as occurred in this patient has been studied and discussed by Albright and his associates.[1] In the present case the patient's single kidney was severely altered as the result of chronic pyelonephritis.[17] His parathyroid glands were enlarged, hypercellular and depleted of fat content. These changes were in keeping with the alterations described in secondary parathyroid hyperplasia of renal origin.[18] The additional finding of numerous "oxyphil" cells, often gathered in clusters, was further evidence that the parathyroid changes were of pathologic significance and did not represent merely variations within the normal limits.[19]

SUMMARY

The anatomic findings in a case of bilateral Legg-Perthes disease of approximately two years' duration have been recorded. The histologic observations made on one hip have been described in detail, and the pertinent findings have been illustrated. The characteristics were those of massive bone and marrow necrosis of the femoral capital epiphysis resulting in marked deformity of the hip joint but causing relatively little damage to the articular cartilage. It appeared probable that these changes had resulted from interference with the blood supply to the epiphysis. Since death supervened before physiologic epiphysial fusion occurred, neither complete organization nor reparative ossification had appeared.

Of additional interest in this case was the severe renal impairment which led to hyperphosphatemia, hyperplasia of the parathyroid glands and acidosis. The last two pathologic states had resulted in renal osteitis fibrosa cystica, formerly termed renal rickets or renal dwarfism.

25 Shattuck Street.

17. Weiss, S., and Parker, F., Jr.: Medicine **18**:221, 1939.
18. Castleman, B., and Mallory, T. B.: Am. J. Path. **13**:553, 1937.
19. Castleman, B., and Mallory, T. B.: Am. J. Path. **11**:1, 1935.

HISTOGENESIS OF THE SO-CALLED GRAWITZ TUMOR

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Stoerk,[1] in his paper "The Histogenesis of Grawitz Tumors," stated that the problem of the interpretation of this tumor is "one of the most investigated chapters of pathology," but he added that "the histogenesis of these tumors still remains an open question." Of these two statements, the latter is still valid, whereas the former has been invalid for the past ten to twenty years.

When Grawitz,[2] in 1883, published the paper in which he traced the origin of the tumor he called "struma lipomatodes aberrata renis" back to cells of the type that form the adrenal cortex, numerous followers supported his theory. But when, in 1908, Stoerk attacked the theory that this tumor (for which Birch-Hirschfeld had meanwhile coined the term "hypernephroma") was of adrenal origin and suggested a nephrogenic orthotopic origin, numerous authors supported the latter theory. Among the pathologists who followed Grawitz' line of thought were Sobolotnow, Lubarsch, Askanazy, Steinke, Chiari, Beneke, Schmorl, Gatti,[3] Kelly, Peham, Winkler, Busse, Pick, Weichselbaum [4] and others. On the other hand, Stoerk's theory, which had previously been suggested by Sudeck,[5] found support from Kaufmann, Horn, Zehbe, Ipsen, Gellé,[5a] Hartung, Lindström, Sisson and others. Grawitz in his first paper [2] failed to give a clear definition of this tumor, which he intended to

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In view of the enormous literature on the topic, only the very important and the more recent papers are quoted extensively. For the earlier references, an almost complete bibliography can be found in F. Henke and O. Lubarsch: Handbuch der speciellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 6, pt. 1.

1. Stoerk, O.: Beitr. z. path. Anat. u. z. allg. Path. **43**:393, 1908.

2. Grawitz, P.: Virchows Arch. f. path. Anat. **93**:39, 1883; Arch. f. klin. Chir. **30**:824, 1884.

3. Gatti, G.: Virchows Arch. f. path. Anat. **144**:467, 1896.

4. Weichselbaum, A., and Greenish, R. W.: Wien. med. Jahrb., 1883, p. 213.

5. Sudeck, P.: Virchows Arch. f. path. Anat. **133**:405, 1893.

5a. Gellé: Bull. et mém. Soc. anat. de Paris **84**:174, 1909.

classify according to its histogenesis. He gave only a more or less accurate description of it as observed in several cases. His mistake was similar to those made by Krukenberg and Brenner, who likewise merely described a few specimens of their newly discovered ovarian tumors without pointing out the characteristics and attributes which are needed in order to classify these tumors into the two new groups. The result of Grawitz' mistake was a flooding of the literature with papers by authors opposing one another.

After the publication of many papers in the years following Grawitz' and Stoerk's presentation of their theories, the dispute gradually subsided and within the last ten years only a few articles have been published on this subject. Unfortunately, however, none of the authors of these recent articles have availed themselves of the great wealth of information which is now recorded concerning the kidneys and the adrenals from histologic and physiologic points of view, for better understanding the histologic nature and the genesis of the hypernephroma. Consequently, today most textbooks of pathology mention both theories, the nephrogenic and the adrenogenic, without making a decision as to which is the correct one.

Corresponding to the standards of pathology at that time, the criteria used by Grawitz, Stoerk and their followers were mostly simple, morphologic ones. Grawitz stressed the polyhedral shape of the tumor cells, the large number of fat or even lipoid droplets in the foamy cytoplasm, the subcapsular location of the small tumors in the upper pole of the kidney and the sharp demarcation from the surrounding renal tissue produced by a well developed capsule. Stoerk, on the other hand, stated that in many instances the initial tumor can be found in the depth of the renal parenchyma far from the capsule, that in many the tumor is found in the middle or the lower part of the kidney far from the upper pole, which is adjacent to the adrenal gland, that the accumulation of fat droplets in the cytoplasm is not specific for the adrenal cortex and can be seen frequently in the tubules of diseased kidneys and that the typical Grawitz tumor cells have hydropic vacuoles without fat.

Grawitz paid special attention to the fact that the hypernephroma has a tendency to store fat in the protoplasm of its large polygonal cells, thus duplicating cells of the adrenal cortex. And when, with progress in biochemistry, the fat droplets of the adrenal cortex had been identified as double-refracting lipoids, later investigators, e. g., Sisson,[6] Zehbe,[7] Werner and Wolfgang Gerlach[8] and Stoerk, postulated that only the

6. Sisson, W. J.: Beitr. z. path. Anat. u. z. allg. Path. **49**:476, 1910.

7. Zehbe, M.: Virchows Arch. f. path. Anat. **201**:150, 1910.

8. Gerlach, Werner, and Gerlach, Wolfgang: Beitr. z. path. Anat. u. z. allg. Path. **60**:383, 1915.

presence of such double-refracting lipoids, and not that of neutral fats, in the hypernephroma should be used to prove adrenal origin. However, since Stoerk, Löhlein and others demonstrated the presence of double-refracting lipoids in pathologic kidneys, especially in cases of nephrosis, this sign has lost some of its diagnostic significance. Zehbe and Lubarsch have stressed the fact that some of the tumors that are doubtlessly nephrogenic have a great tendency for fatty degeneration.

For all these reasons only a convincing similarity between the tumor cells and the cells of the adrenal cortex (polyhedral shape, densely arranged lipoid droplets, leaving only a thin network of pale-staining protoplasm, and a centrally located, small, shrunken, dark nucleus) should be used as a criterion to identify tumor cells as arising from cells such as form the adrenal cortex.

Stoerk and his followers attached great significance to the following two contentions: (1) that the parenchyma of the Grawitz tumor forms lumens and may even become papillomatous, a tendency and a potency which the adrenal cortex and its tumors definitely do not have, and (2) an important observation by the Gerlachs and by Trotter and Hartung, that neither the adrenal cortex itself nor the adrenal cortical islands which spread from the kidney to the pelvis ever have given origin to real Grawitz tumors. Today one knows that the second statement is not true, for among the cases of so-called hypernephroma of the ovary reviewed by Levy du Pan, several cases were described in which the type of tumor cells, the formation of lumens and the papillary growth duplicated the findings in the true Grawitz tumor (for instance, the case published by Rosthorn [8a] in 1909; fig. 1 *A*).

Many authors agree that the Grawitz tumor develops from the well known renal papilloma which is found frequently and sometimes in multiple form in sclerotic kidneys and that the cellular elements of the papilloma can be traced back to their origin from the epithelium of the involuted tubules. Included among these authors are Ewing, Zehbe, Frattini, Kaufmann, Lubarsch, Lissard, Nürnberg,[9] Borst, Sisson, Ipsen and Stroebe.[10]

In general, the convoluted tubules first develop into cysts; these cysts then become papillomatous, and the papillomatous structures give rise to Grawitz tumors. In a small minority of cases, however, the cortical papillomas do not originate from cysts by papillary proliferation of the epithelium but develop directly from the epithelium of the convoluted tubules without the latter undergoing cyst formation. In such cases the tumor growth is a multicentric one, and the demarcation from the sur-

8a. von Rosthorn, A.: Verhandl. d. deutsch. Gesellsch. f. Gynäk. **13**:362, 1909.
9. Nürnberg: Frankfurt. Ztschr. f. Path. **1**:433, 1907.
10. Stroebe: Centralbl. f. allg. Path. u. path. Anat. **6**:720, 1895.

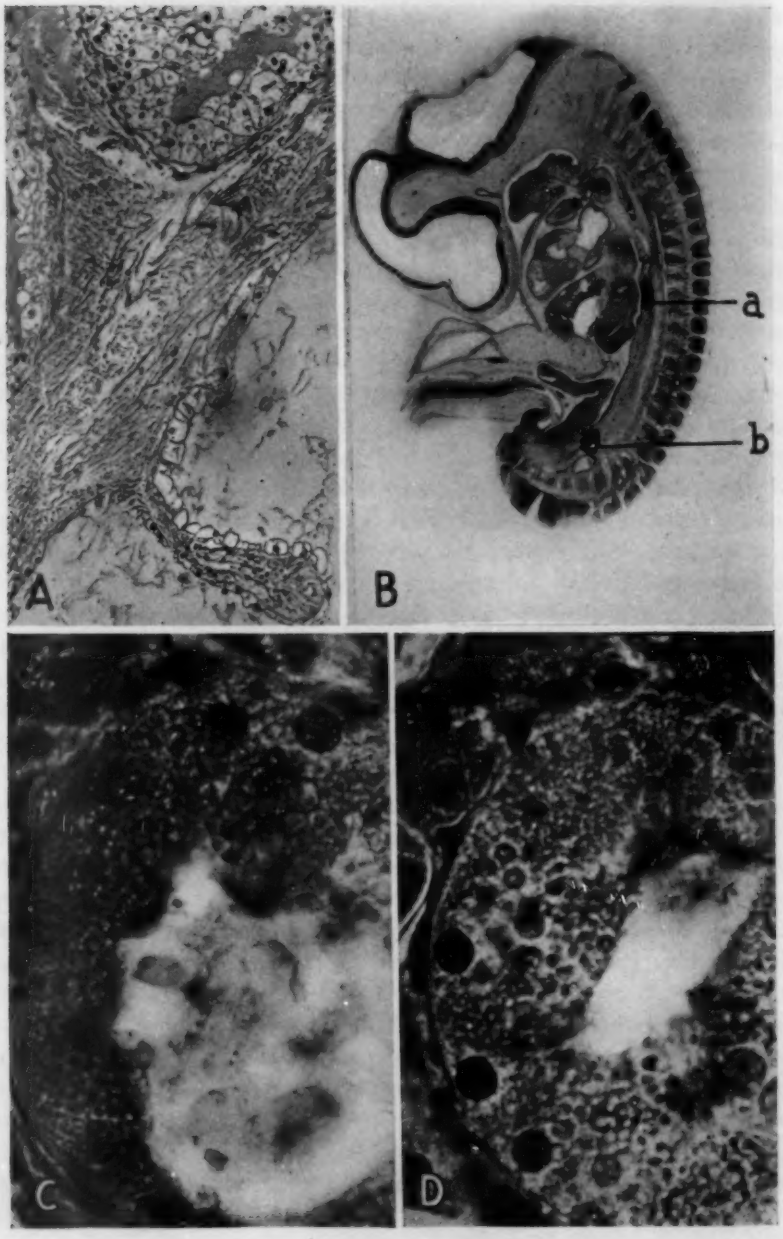

Figure 1

(See legend on opposite page)

rounding normal tissue is very indistinct. In these cases the difference between the tumor cell and the normal tubular cell is the same as in the papillomas which originate from cortical cysts: The tumor cell is larger, and the nucleus covers a larger part of the cell and stains deeper. Sometimes in one and the same kidney numerous papillomatous cysts and a multicentric papilloma arising from the convoluted tubules can be found.

When, on the other hand, the adrenal cortical character and origin of the Grawitz tumor are conceded, the development of this tumor from the renal tubular epithelium necessitates the formulation of a hypothesis that among the cellular elements of the involuted tubules there are misplaced adrenal cortical cells. This hypothesis seems possible in the cases in which the tumor arises in the upper pole of the kidney, which, of course, is adjacent to the adrenal gland, but does not appear very possible in the case of those papillomas or tumors which originate in the middle or lower part of the kidney. Although so-called misplaced adrenal tissue has been seen in the pyramids of the kidney (Sonneberg [11]), such observations are very rare and the correctness of their interpretation is doubtful. Robert Meyer in his classic paper on *Keimversprengung* (germ dispersion) expressed a well founded warning against the explanation of heterotopic tissue or heterotopic neoplasms by simple dislocation of fetal tissue. This explanation is to be accepted only if a tissue during fetal life can through normal development reach a remote area by "illegal, abnormal connection" with an adjacent tissue in such a manner that this growing tissue carries the neighboring resting cells into areas in which the latter cells normally never settle. This is the only mechanism of real "fetal dislocation," and whenever "illegal fetal connection" of an extending growing tissue and a neighboring resting tissue cannot be assumed, the heterotopic presence of the latter tissue should not be explained by mechanical dislocation. Simple inclusions of parts of an

11. Sonneberg, E. A.: Ein Fall von Versprengung von Nebennierengewebe in die Papillenspitzen der Niere, Inaug. Dissert., Munich, 1910.

EXPLANATION OF FIGURE 1

A, hypernephroma of the ovary, adenopapillary structure (case of Rosthorn). *B*, human embryo at the fifth week, 10 mm. in greatest length (collection of Dr. P. Gruenwald). The location of the primordium of the adrenal cortex is shown at *a*; the site of the primordium of the metanephros (permanent kidney) at *b*. *C*, tubule with hyaline droplets, particularly in the supranuclear zone. (The patient was a 23 year old white man with amyloid nephrosis and chronic suppurative sacroiliac arthritis.) *D*, tubule from the borderline zone of an anemic infarct; the epithelial cells are loaded with hyaline droplets, particularly in the supranuclear zone. (The patient was a 49 year old white man with rheumatic endocarditis, benign nephrosclerosis and anemic infarcts of the left kidney.)

adjacent tissue are entirely different from dislocations of distant tissue and can be explained on the basis of incomplete or faulty demarcation of neighboring organs. Such mistakes in demarcation can be accepted, however, only in the case of organs which primarily develop from a common parenchyma and later become separated by a secondary demarcation—for example, the ovary and the mesonephros. If the separating folds did not develop exactly between these two tissues but within the mesonephric tissue, it is obvious that this faulty demarcation would result in the incorporation of mesonephric cells in the field of the ovarian parenchyma. These potential mesonephric cells may later give rise to an ovarian tumor of renal character, the mesonephroma. However, such a mechanism is possible only in organs which develop adjacent to each other and from a common parenchyma.

The metanephros and the adrenal gland develop far from each other and come in contact only secondarily and comparatively late, when each already has a well developed surface capsule [12] (fig. 1 *B*). Thus the mechanisms of "error in demarcation" or "misplacement of cells" should not be considered as possible causes for the formation of Grawitz tumors, not even for one occurring in the upper pole of the kidney. This is substantiated in the statistics gathered by Ipsen, Wilson and Willis,[13] Hefke and Küster, which reveal that "adrenal inclusions" are found no more frequently in the upper pole of the kidney than in any other part of the kidney. (Of 194 cases of Grawitz tumor, the middle portion of the kidney was involved in 80, the lower portion in 60 and the upper pole in 54.)

Today there is evidence that during fetal development cells having adrenal cortical potencies are spread along the entire urogenital fold, extending down to the epididymis or to the ovarian hilus. In the hilus of the human ovary and in the human epididymis one occasionally finds groups of cells possessing adrenal cortical character, a finding which is more frequent in the rabbit. Whether adrenal potencies are active in all the cells of the kidney during its development, or are physiologically inactive except in a few cells, or remain latent in all the cells and become activated by chance remains a question.

Thus, one may see that even the best and most flexible theory, the hypothesis of the Gerlachs, that the Grawitz tumor develops from misplaced fetal adrenal elements which become fused with fetal renal

12. The capsule of the kidney dates back to the latter part of the second month. At this time the kidneys and the adrenals are still separated by a considerable distance. The two organs meet each other, but not before the middle of the third month. This contact is established on the left side earlier than on the right. At the time of contact each gland possesses already a well developed capsule which would prevent misplacing of its tissue by mechanical mingling.

13. Wilson, L. B., and Willis, B. C.: J. M. Research **24**:73, 1911.

elements thereby giving rise to a tumor which has the potency of developing into both types of tissue, does not explain the development of a Grawitz tumor far from the upper pole of the kidney.

Some authors, as Lubarsch,[14] Manasse,[15] Nürnberg, Ricker,[16] Bailey and Harrison [17] and Stroebe, have tried to determine the origin of the Grawitz tumor rather by cytologic characters than by histologic ones. But the minute cytologic differences in the distribution of chromosomes or in the relative sizes of the nucleoli are very unstable criteria in so polymorphous a tumor as the Grawitz tumor. Moreover, these cytologic findings change very rapidly with neoplastic transformation of normal tissue and even more rapidly with malignant degeneration of benign tumors.

It is obvious, therefore, that contradictions and discrepancies make it difficult to establish either a common histogenesis or a simple classification for all Grawitz tumors. Many authors have attempted to establish a classification of these tumors, as the Gerlachs, Bell,[18] Judd,[19] Hefke, Lindström,[20] Rosenfeld,[21] Wilson and Willis, Hansemann, Nürnberg and Ricker. However, in using one of these classifications it has been necessary to classify a large number of cases under the heading of "atypical." This can mean only that either these cases do not fit into the system or the histogenetic foundation of this classification does not offer space for all the cases. Needless to say, such a classification should not be accepted as final.

Most of the investigations concerning Grawitz' theory of adrenal and Stoerk's theory of renal origin of the Grawitz tumor were carried out at a time when the kidney was considered solely as an excretory organ. Approximately twenty years ago physiologists discovered that the main role of the renal tubule is reabsorption and not excretion as was previously thought. The reabsorption of substances excreted by the glomeruli may be complete (e. g., sugar) or incomplete (e. g., water). Only a few substances (e. g., urea) are excreted and not absorbed by the renal tubules. Tubular reabsorption and excretion, however, vary in different animals; substances which are absorbed in one species are excreted in another and vice versa. Shannon,[22] in an excellent article, has given a general review of the great varieties of tubular functions. Renal tubular activity has been investigated and studied for many physiologic sub-

14. Lubarsch, O.: Virchows Arch. f. path. Anat. **135**:150, 1894.
15. Manasse, P.: Frankfurt. Ztschr. f. Path. **11**:403, 1912.
16. Ricker, G.: Centralbl. f. allg. Path. u. path. Anat. **8**:417, 1897.
17. Bailey, O. T., and Harrison, J. H.: J. Urol. **38**:509, 1937.
18. Bell, E. T.: J. Urol, **39**:238, 1938.
19. Judd, E. S., and Hand, J. R.: J. Urol, **22**:10, 1929.
20. Lindström, L. J.: Arb. a. d. path. Inst. d. Univ. Helsingfors **2**:299, 1921.
21. Rosenfeld, E.: Frankfurt. Ztschr. f. Path. **14**:151, 1913.
22. Shannon, J. A.: Physiol. Rev. **19**:63, 1939.

stances (e. g., water, urea, creatinine, dextrose, uric acid) and for many test substances (e. g., neutral red, xylose, sucrose, inulin) in human subjects and also in many animals. Some authors still question the exact nature of the reaction of the renal tubules to protein. This question is of great importance in renal pathology, especially in the pathology of nephrosis. Only when one knows the origin of the large quantities of protein excreted in nephrosis can one hope to have an accurate conception of the genesis of this disease.

Vollhard and Fahr, who first conceived of nephrosis as an entity, separate and distinct from nephritis, explained the abundance of protein in the urine on the basis of an excretory dysfunction of the degenerated renal tubules. Fahr's conception found support through Lichtwitz, Kosugi, Benoit, Lascano and Gonzalez. On the other hand, Randerath [23] and his school traced the origin of this protein to the glomeruli and expressed the belief that the tubules were responsible for the partial absorption rather than for the secretion of this protein.

It was the occurrence of a special pathologic change in the renal tubular epithelium in nephrosis and renal amyloidosis and in the border zone of anemic infarcts which aroused a great deal of interest. This change consisted of the formation of so-called hyaline droplets; i. e., the renal tubular epithelium was found to be stuffed with highly refractile globular droplets of dense hyalin which stained deeply with acid dyes, especially with eosin (fig. 1 *C* and *D*). Three explanations were offered for the origin of these droplets: (1) secretion from the plasma of the blood in the interstitial capillaries, (2) absorption of protein in the glomerular secretion by the renal tubular epithelium or (3) degeneration of the protoplasm of the renal tubular cell. Fahr [24] and his school adopted the first explanation, namely, that of secretion, and accepted degeneration as a possibility only for those droplets which were irregular in size and distribution. Randerath, on the other hand, adopted the second explanation, namely, that of absorption and gave the teleologic explanation that the tubules attempt to conserve for the organism that protein which has been cast away by the glomeruli.

Experiments were performed to determine the conditions under which hyaline droplets develop in the kidneys of animals, particularly of urodeles, amphibia and small rodents. The results of these experiments have been published by Laas,[25] Lambert,[26] Terbruggen,[27] Suzuki, Newmann and Whipple, Gérard and Cordier and others. The most con-

23. Randerath, E.: Beitr. z. path. Anat. u. z. allg. Path. **95**:403, 1935.

24. Fahr, T.: Ztschr. f. klin. Med. **134**:533, 1938; Klin. Wchnschr. **26**:1205, 1931.

25. Laas, E.: Virchows Arch. f. path. Anat. **286**:426, 1932.

26. Lambert, P. P.: Beitr. z. path. Anat. u. z. allg. Path. **98**:103, 1936.

27. Terbruggen, A.: Virchows Arch. f. path. Anat. **290**:574, 1933.

vincing results were obtained by Lambert, who used urodeles as the experimental animals. These animals have two different types of nephrons: those which are closed by glomeruli and those which are open and communicate with the peritoneal cavity. Two different proteins were used in these experiments: one having a low molecular weight, for which the glomerular wall is permeable, and the other having a high molecular weight, for which the glomerular wall is impermeable. The proteins of high molecular weight produced hyaline droplets only in the open nephrons and then only when administered intraperitoneally. The proteins of low molecular weight produced hyaline droplets in both the open and the closed nephrons regardless of whether administered hypodermically or intraperitoneally. These findings prove that the hyaline droplets originate from the protein which is present in the glomerular filtrate in the lumens of the renal tubules and not from the protein which circulates in the interstitial capillaries.

The conclusion that the hyaline droplets found in the renal tubular epithelium in nephrosis originate from the glomerular filtrate harmonizes well with the distribution of these droplets. They are found in largest numbers in the proximal portion of the tubule, i. e., the part closest to the glomerulus,[28] and in the upper part of the cell, i. e., that portion of the cell which borders the lumen, not the basal portion, which is adjacent to the interstitial capillaries. Moreover, these droplets are never found passing from the cells into the lumen nor are they ever present within the lumen itself.

This conclusion also supports the theory of tubular reabsorption and confirms the suggestion of Bell and others that the principal pathologic change in the nephrotic kidney is the increased permeability of the glomerular loops for proteins.

Hyaline degeneration characterized by irregularly distributed granules of different sizes can be found in epithelium of other organs and in other cells (e. g., plasma cells, according to Apitz) as well as in the kidney, especially in the distal portion of the renal tubules. This picture, however, differs considerably from that already described.

Hyaline degeneration of the cells in Grawitz tumors has already been observed by Zehbe, Lubarsch and even by Stoerk. But at the time that this observation was made, the entity of nephrosis and the problem of nephrotic albuminuria and of nephrotic hyaline tubular degeneration were not yet known. When I revised and studied my material on the Grawitz tumor, I was amazed by the close resemblance of some cells

28. In general, the hyaline droplets are more numerous in the proximal than in the distal portions of the convoluted tubules. Sometimes they are present only in the proximal portions. In a case of stricture of the rectum as a result of lymphogranuloma venereum in a young Negro woman, I found hyaline droplets only in that portion of the tubule which was directly attached to the glomerulus.

containing hyaline droplets in the Grawitz tumor to cells in kidneys with nephrosis, amyloidosis or infarcts. The same type, size, shape, density and coloring of the hyaline droplets, as well as the same limitation of distribution (i. e., to the upper portion of the cytoplasm), could be seen (fig. 2, *A* and *B*). The localization of the hyaline droplet within the tumor is illuminating from the pathogenic standpoint. These droplets are found only in those cells which line a lumen and never in the solid portions of the tumor. If one finds in an apparently solid portion of the tumor cells carrying masses of hyaline droplets along the upper border, careful examination of the slide will reveal a small crescent-shaped lumen. Wherever hyaline droplets are present, the lumen contains thick, easily stainable proteinic fluid. The hyaline droplets never appear in the lumens of cystic cavities nor can they be found passing through the upper cellular border. The cells containing hyaline droplets are anchored, like other epithelial cells, in the connective tissue of the septums and have no special topographic relation to the interstitial vessels. Usually these cells are separated from the neighboring capillary by a thick layer of fibrous tissue (fig. 2, *A*).

The findings described permit the following conclusions: (1) that the hyaline droplets found in the Grawitz tumor and those found in the renal tubular epithelium originate from the protein in the lumen and not from the protein in the blood (i. e., the findings substantiate the theory of absorption and abolish the theory of secretion, as well as the theory of local cytoplasmic degeneration), (2) that the hyaline droplets are absorbed and not excreted by the renal tubules [29] (as revealed by the close analogy between the findings described and those obtained by animal experimentation) and (3) that the Grawitz tumor is of renal character because the described hyaline droplets are specific for the kidney.

A functional structure like hyaline droplets in a tumor where the tissue has no physiologic relation to other organs and no coordination with the general nervous, vascular or hormonic regulation, and no functional control, reveals how great the functional independence of a tissue can be. Other examples of functioning tumors are the hornifying car-

29. Although the theory of excretion can be taken into consideration as a possible explanation for the hyaline origin of hyaline droplets in both nephrosis and amyloidosis because in both of these conditions there are abnormal proteins circulating in the plasma, this explanation meets with difficulties in the case of the anemic infarct. Here the abnormal proteins which originate from the decomposed parenchyma of the infarct could possibly reach the affected tubules from the luminal side, provided that the glomerulus lies within the infarcted area but is still viable because of an independent blood supply. It certainly is not likely under these circumstances that the protein reaches the tubular epithelium from the interstitial capillary side, since the vessels leaving the infarcted area are no longer functioning.

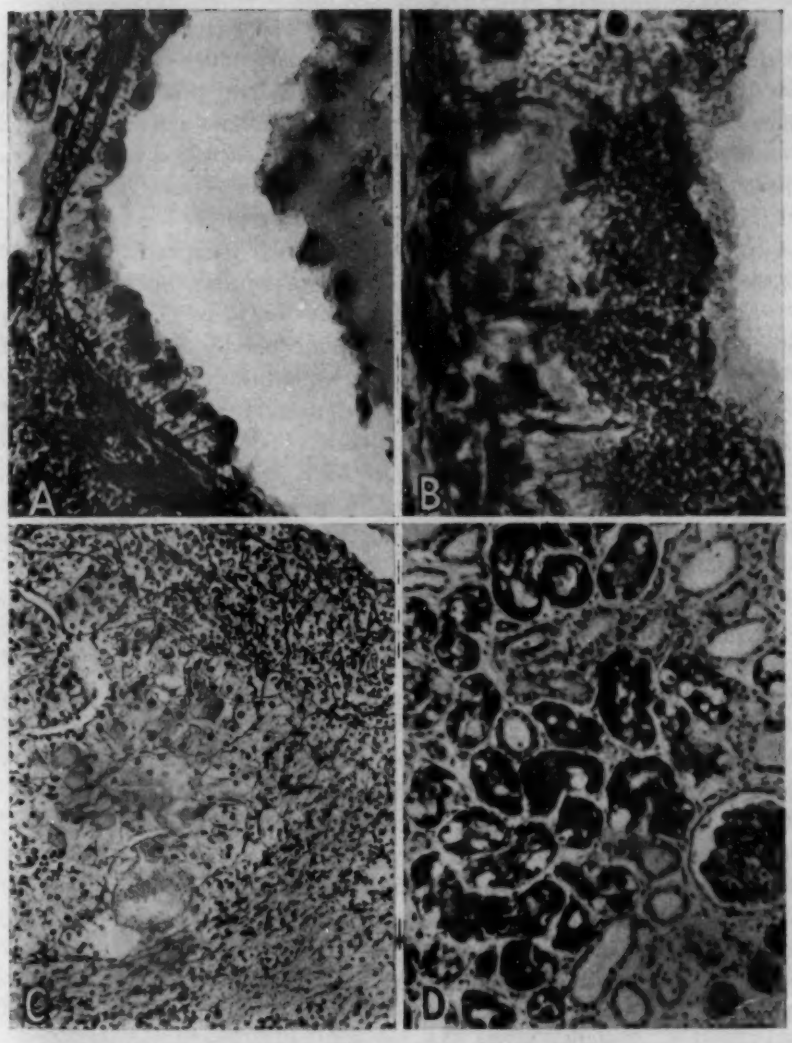

Fig. 2.—*A*, Grawitz tumor—high columnar epithelium lining a cavity which contains thick proteinic fluid; the supranuclear part of the protoplasm is loaded with hyaline droplets. (The patient was a 45 year old white woman with progressive recurrence of a Grawitz tumor after operation.) *B*, the same epithelium under high magnification. *C*, the solid part of the tumor; the cells are without hyaline droplets. *D*, storage of hematogenic iron pigment in the convoluted tubules. (The patient was an 18 year old Negro youth with sickle cell anemia and severe jaundice after splenectomy.) Berlin blue reaction; nuclear-fast red staining.

cinoma of the skin, the mucus-producing carcinoma of the intestinal mucous membrane and the various tumors with endocrine activities (e. g., neoplasms of the thyroid or the pancreas, dysfunction in arrhenoblastoma of the ovary and virilizing carcinoma of the adrenal).

Chambers and Cameron [30] and Chambers and Kempton [31] have found that in tissue cultures fragments of the proximal tubules of the mesonephros of the chick embryo if kept viable for a long time come to have closed ends and distended lumens as a result of cellular activity. If phenol red is added to the medium, it accumulates by secretion in the lumen, thereby proving the functional activity of tubular epithelium which is neither connected with a glomerulus nor under physiologic nervous or vascular control. By a similar autonomic cellular independence, the cells of the Grawitz tumor store hyalin by taking it from the fluid in the lumen.

Another protein of the blood, whose relation to tubular function has proved to be of great importance in the understanding of tubular physiology, is hemoglobin. The mechanism of hematuria has been investigated by numerous authors; among them are Adami, Silbermann, Miller,[32] Baehr, Schmidt, Sellards and Minot, Böhm, Masius, Lebedeff, Bostroem, Fraenkel, Kahlen and Emrich. However, it was Ribbert who, in 1896, stated for the first time that in hematuria the hemoglobin is excreted by the glomerulus. Adami agreed with Ribbert, whereas Silbermann, as well as Miller, spoke of tubular secretion of hemoglobin. But Baehr, in 1913, gave an explanation which anticipated the recent conception: filtration by the glomerulus and storage by the tubule. In an experimental paper Fukuda and Oliver [33] offered two explanations for the increased concentration of hemoglobin in the tubules: Either there was absorption of water or there was additional excretion. They finally decided that the second was the "more efficient and practical" explanation. After the most illuminating discoveries of H. Smith, who demonstrated the phylogenetically acquired and preserved, functionally very unpracticable mechanisms of the human kidney, this manner of teleologic reasoning cannot be accepted any more. The decisive investigations of this problem were carried out by Klingmüller [34] and particularly by Lisson.[35] The latter used toads for his experiments. In these animals the glomerulus and the tubulus get their blood supply from different arteries. Parenteral injection of hemoglobin produces hematuria and storage of hemoglobin in the tubular epithelium. This storage is not found if prior to the administration of the hemoglobin the glomerular

30. Chambers, R., and Cameron, G.: J. Cell. & Comp. Physiol. **2**:99, 1932.
31. Chambers, R. and Kempton, R. T.: J. Cell. & Comp. Physiol. **3**:131, 1933.
32. Miller, J. W.: Frankfurt. Ztschr. f. Path. **11**:403, 1912.
33. Fukuda, Y., and Oliver, J.: J. Exper. Med. **37**:83, 1923.
34. Klingmüller, K.: Ztschr. f. d. ges. exper. Med. **103**:106, 1938.
35. Lisson, L.: Beitr. z. path. Anat. u. z. allg. Path. **101**:94, 1938.

blood supply is cut off (as, for example, by tying the glomerular artery). The same results can be achieved in spite of the tied glomerular artery when the hemoglobin solution is injected directly into Bowman's glomerular space. Lisson summarizes his findings by the words: Without glomerular filtration, neither hematuria nor tubular storage of hemoglobin can occur. Hemoglobin evidently is stored by the tubules from the filtrate in the lumen and not from the plasma of the blood (fig. 2 *D*). The mechanism is the same as that in the formation and storage of hyaline droplets. Vital dyes like pyrrole or trypan blue are stored in a similar way, as little droplets in the upper part of the cytoplasm of the tubular epithelium. This was demonstrated by Hayman and Richards, Tannenberg and Winter, Gérard and Cordier (fig. 3 *A*). Möllendorff, who has done a considerable amount of work on this topic, summarized his observations by stating that the various findings can be explained only by assuming that the dyes are absorbed by the tubular epithelium from the glomerular filtrate in the lumen. The faculty to absorb and to store hemoglobin is in general found only in the cells of the reticuloendothelial system and not in epithelial cells. I have examined a great number of normal epitheliums in the vicinity of hematomas and also of neoplastic epitheliums in tumors with hemorrhagic necroses (especially ovarian cystomas and papillomas, and intestinal polyps) and am convinced that phagocytosis or absorption and storage of hemoglobin, a faculty which is almost monopolized by the reticuloendothelial system and the interstitial elements, are never practically shared by epitheliums. However, there are a few exceptions—for example, the epitheliums of the liver, thyroid, pancreas and adrenal cortex in cases of hemochromatosis. Two explanations are possible: Either the serum contains so large a quantity of hemoglobin that the cells become flooded and saturated with it, or else there is toxic damage to the epitheliums which permits the imbibition of hemoglobin. It is interesting that in such cases the kidneys may be completely free from blood pigment. This proves that there is neither renal hemoglobinuria nor hematuria as long as the glomeruli are intact and that only glomerular pathologic permeability for hemoglobin is the cause of this pathologic excretion. The passive character of the imbibition shown by the cells of the liver and other organs in hemochromatosis can be proved by the distribution of the granules all over the cytoplasm. The storage only in the upper part of the cell is found only with tubular absorption from the lumen. Grawitz tumors generally are well vascularized, and since this specific tissue is prone to decomposition and necrosis, hemorrhages are found very frequently. Wherever free decomposing red blood corpuscles come in contact with tumor cells lining the wall of the cavity, absorption of hemoglobin by the tumor cells can be observed. The size, the number and the density of the absorbed droplets present great variations.

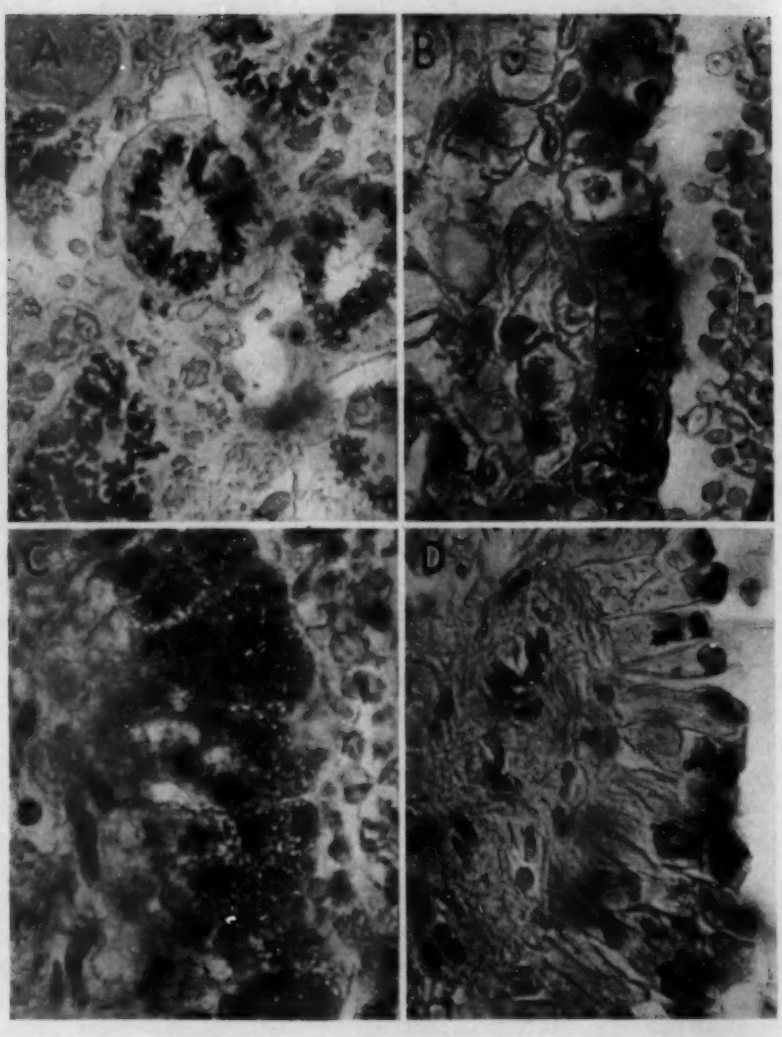

Fig. 3.—*A*, renal tubules of a white mouse one day after an intraperitoneal injection of 1 cc. of a 2 per cent watery trypan blue solution. Note the storage of dye droplets in the supranuclear zone of the tubular epithelium. *B*, Grawitz tumor of the right kidney, 18 by 30 by 16 mm., sharply demarcated, yellow with hemorrhagic areas. The cuboidal epithelium adjacent to a fresh hematoma has stored hematogenic iron pigment granules. (The patient was a 60 year old white man with a hypertensive heart.) Berlin blue reaction; nuclear fast red staining. *C*, high columnar epithelium of a papillary adenoma of the renal cortex (12 by 16 by 10 mm.), the supranuclear part of the protoplasm loaded with hemoglobin granules from red blood corpuscles of a fresh hematoma in the lumen. (The patient was a 58 year old Negro man with syphilitic mesaortitis and a diffuse aneurysm of the ascending aorta.) *D* (same case as *C*), papillary projection of the adenoma. The columnar epithelium contains granules of hematogenic iron pigment. Turnbull blue reaction; nuclear fast red staining.

Sometimes the cytoplasm is almost replaced by dense masses of small granules; other times only a few large round hemoglobin droplets can be seen. Storage of these granules takes place only in the upper part of the cell; the lower basal part, i. e., the infranuclear zone, remains free. This picture duplicates exactly the findings in experimental and in clinical hematuria and proves the renal tubular character of the cells (fig. 3 *B, C* and *D*).

Epithelial storage of hemoglobin is so specific for renal tubular cells that it may be used under certain circumstances for the identification of a questionable tissue. Beneath the basal part of the capsule of the left lateral lobe of the prostate gland of a Negro of 46 years, who died of malignant nephrosclerosis, I found at the microscopic examination a round cystic cavity, 3.5 mm. in diameter. It was lined with low racemous papillary projections having an epithelium which was different from the normal columnar prostatic epithelium. The low cuboidal cells with pale vesicular nuclei resembled tubular epithelium. In the center of the little cyst was a mass of free and of clotted red blood corpuscles. Almost all the epithelial cells lining the cystic cavity had relatively large round droplets of hemoglobin in their protoplasm. The septums contained hemosiderin, which gave a positive reaction for iron (fig. 4 *A* and *B*). The absorption of hemoglobin supports the conclusion that the little cyst represents heterotopic renal tubular tissue, comparable to a similar observation by Robert Meyer, who once found an isolated glomerulus in the pelvis.

The absorption of hyaline droplets and the storage of hemoglobin, being specific for renal tubular structures, confirm the renal tubule origin of the Grawitz tumor cell (fig. 4 *C*). Thus far these findings fit in well with the histogenic conception that the tumor usually originates from cortical papillomas, and cortical papillomas develop from cortical cysts, and the cysts develop from blocked tubular elements. The mechanical isolation of parts of a tubular loop by shrinkage of the neighboring tissue occurs more frequently in the subcapsular region and with greater probability in protracted shrinkage of the kidney. Consequently one finds a higher incidence of cortical papillomas in sclerotic kidneys and, in such kidneys, mainly beneath the capsule. The localization accounts also for the absence or the presence of a capsule. Some authors, as Aschoff, Lubarsch and Petrenz, use the absence of a capsule and the absence of compression of the surrounding tissue as proof that the little tumors develop by dysontogenesis and replace normal renal parenchyma. Other investigators, e. g., Bailey, Willson and Willis, and Kaufmann, use the presence of a capsule as an argument for the true neoplastic and eventually heterotopic character of the tissue. Stoerk and Rehberg pointed to the fact that small tumors in general have no capsules, whereas larger

ones usually acquire distinct fibrous or even scarlike zones of demarcation. I have examined the border zones of numerous small papillomas of different localization. Those which are localized beneath the cortical surface almost never have capsules. They develop in an area of the kidney where the interstitial fibrous tissue is scarce and consequently not apt to react by forming a demarcating capsule even when the tumor grows larger. The few papillomas which develop in the deeper layers

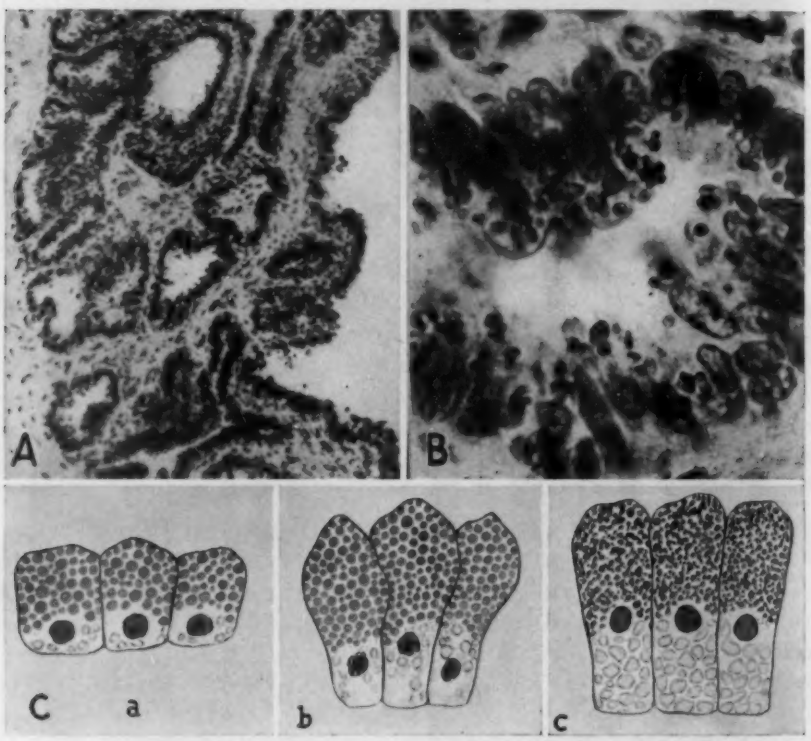

Fig. 4.—Adenopapillary cystic node of the prostate gland; the low columnar epithelium has stored hematogenic iron granules from a hematoma in the lumen. *A*, low power magnification; *B*, high power magnification. (The patient was a 46 year old Negro man with benign nephrosclerosis and cardiac decompensation.) *C*, comparison of cells of a nephrotic kidney with cells of a Grawitz tumor: (*a*) three cells from a tubule in a case of nephrosis, with hyaline droplets; (*b*) three cells from a Grawitz tumor, with hyaline droplets (proteinic fluid in the lumen); (*c*) three cells from a Grawitz tumor with hemoglobin granules (blood in the lumen).

of the cortex and even close to the pyramids develop in an area of the kidney which contains more interstitial fibers and consequently is more apt to furnish a capsule. This explains why papillomas when they grow

beneath the renal capsule border the surrounding tissue directly, without a separating capsule. The deeper in the cortex they lie the more numerous are the interstitial fibers of the surrounding parenchyma and the thicker is the demarcating capsule once the papilloma grows larger. In sclerotic kidneys with multiple papillomas the difference between superficial papillomas without any capsules and deep-seated papillomas with well developed capsules, both sometimes to be seen in one field under low magnification, is very illuminating. These observations of course hold for papillomas which develop secondarily from cysts. Papillomas which originate by multicentric papillomatous proliferations from a small cortical area have no primary clearcut demarcation and, consequently, no capsules in the early phases of their genesis (fig. 5).

So far the renal origin and character of the Grawitz tumor seem to be well established, but there cannot be any doubt that some of the tumors present distinct areas, in the primary growth and in the deposits, which perfectly duplicate the cellular character of the adrenal cortex by forming solid masses of large polyhedral cells with lipoid droplets in the foamy cytoplasm and with small shrunken nuclei, arranged in small groups between thin septums, which consist almost only of thin-walled capillaries. Even if one admits that cystic or papillomatous structure speaks against an adrenal character and that the presence of large light fat-laden cells is not sufficient to prove an adrenal origin, the cytologic and histologic picture of some solid tumors must be accepted as typical of an adrenal cortex type. These cases evidently furnished the material on which Grawitz founded his theory, whereas Stoerk's cases belonged to the larger nephrogenic group. Only a few authors have tried to do justice to the existence of tumors of both groups. Adami,[36] in 1908, said: "A large number of renal tumors are hypernephromas; others are of renal origin." Wilson [37] and Wilson and Willis, who had in their material only adrenal tissue, which was separated from the renal parenchyma by a thick capsule, thus failed to investigate the true "Grawitz" nodules. Their theory that the Grawitz tumor originates from primitive renal blastema which in fetal time did not come in touch with the organizing ureter buds and that it does not originate from adult renal tissue cannot explain the high incidence of adenomatous papillomas in sclerotic kidneys. Their suggestion that the Grawitz tumor be called mesothelioma since it originates from mesodermal parenchyma would, if accepted, force the same name on carcinoma of the mucosa of the fallopian tube and of endometrium, since both of these mucous membranes originate from mesodermal celomatous tissue. The Gerlachs traced the Grawitz tumor back to a misplacement of potential adrenal cells into the

36. Adami, J. G.: Principles of Pathology, Philadelphia, Lea & Febiger, 1908.
37. Wilson, L. B.: Ann. Surg. **57**:522, 1913.

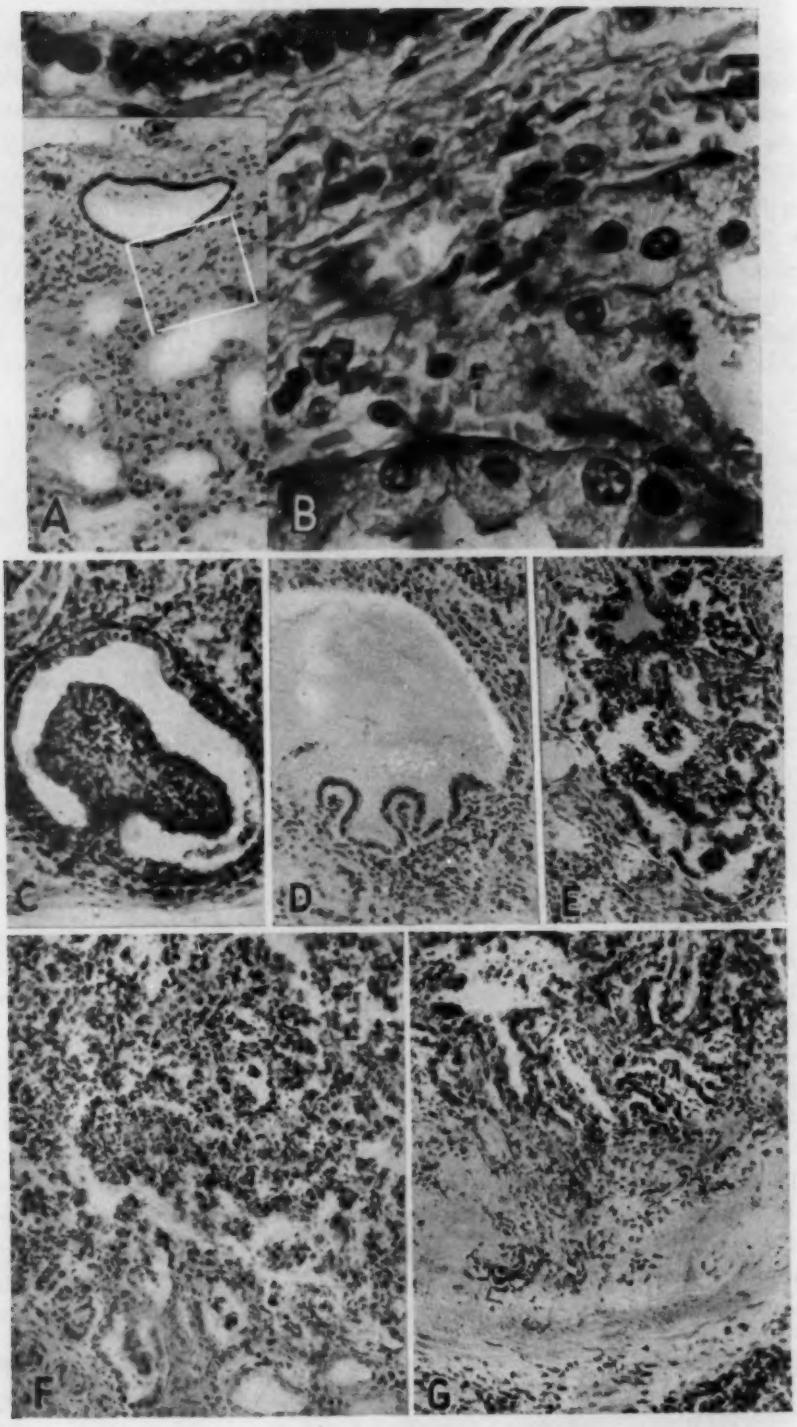

Figure 5

(See legend on opposite page)

renal blastema and a resultant mingling of these cells with potential renal cells. The mixture of potential adrenal and potential renal cells remains undifferentiated as a potential tumor germ in the sense of Cohnheim. It may later, if aroused, give origin to either renal or adrenal tumor tissue. This explanation does injustice to the conception of misplaced fetal tissue and lacks direct histologic evidence. The conception does not explain the transitions from one type into the other which frequently can be observed. Most of the early small papillomas present neither adrenal characteristics nor adrenal cells. It is only during the process of proliferation that adrenal characteristics gradually appear. If the tumors originated from a mixture of both cell types, then even in small tumors one would expect to find both renal and adrenal elements present together in such an arrangement as to prove independent origin of both. Only the findings in the initial phases of developing tumors can be expected to be illuminating and illustrative from the histogenic standpoint. Once a tumor has reached a somewhat progressed phase of growth and development, its component tissues or its tissue has undergone so many marked changes that conclusions as to the origin or the histogenesis are hardly possible.

Among a great number of small, young cortical papillomas I found a few which showed typical adrenal elements. These adrenal cells never lined the wall of the cavity, nor did they cover papillary projections. Only when the lining epithelium becomes piled up as a result of proliferation so as to form solid clusters do the cells assume the typical adrenal character. As long as they line the wall of a cavity and retain their polarity they never possess adrenal cell characteristics. This holds

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EXPLANATION OF FIGURE 5

A, subcapsular renal cortical cyst (at top) and renal tubules (at base) under low power magnification. (The patient was a 59 year old white man with coronary sclerosis.)

B, the same as *A* under high power magnification. At top: epithelium of the cortical cyst with smaller cells and smaller, darker staining nuclei as compared with the tubular epithelium at the base.

C, small renal cortical cyst with beginning papillomatous proliferation. (The patient was a 52 year old white man with chronic bilateral progressive nephrosclerosis and pyelonephritis.)

D, renal cortical cysts with multiple papillary projections. (The patient was a 48 year old Negro man with pyelonephritis and perinephritis.)

E (same case as *D*), renal cortical cyst with progressive papillomatous transformation.

F (same case as *D*), subcapsular renal cortical papilloma without sharp demarcation from surrounding cortical tissue.

G (same case as *D*), papilloma from the cortex of the kidney shown in *F* but from a deeper part of the cortex, close to the pyramids, with a thick fibrous capsule.

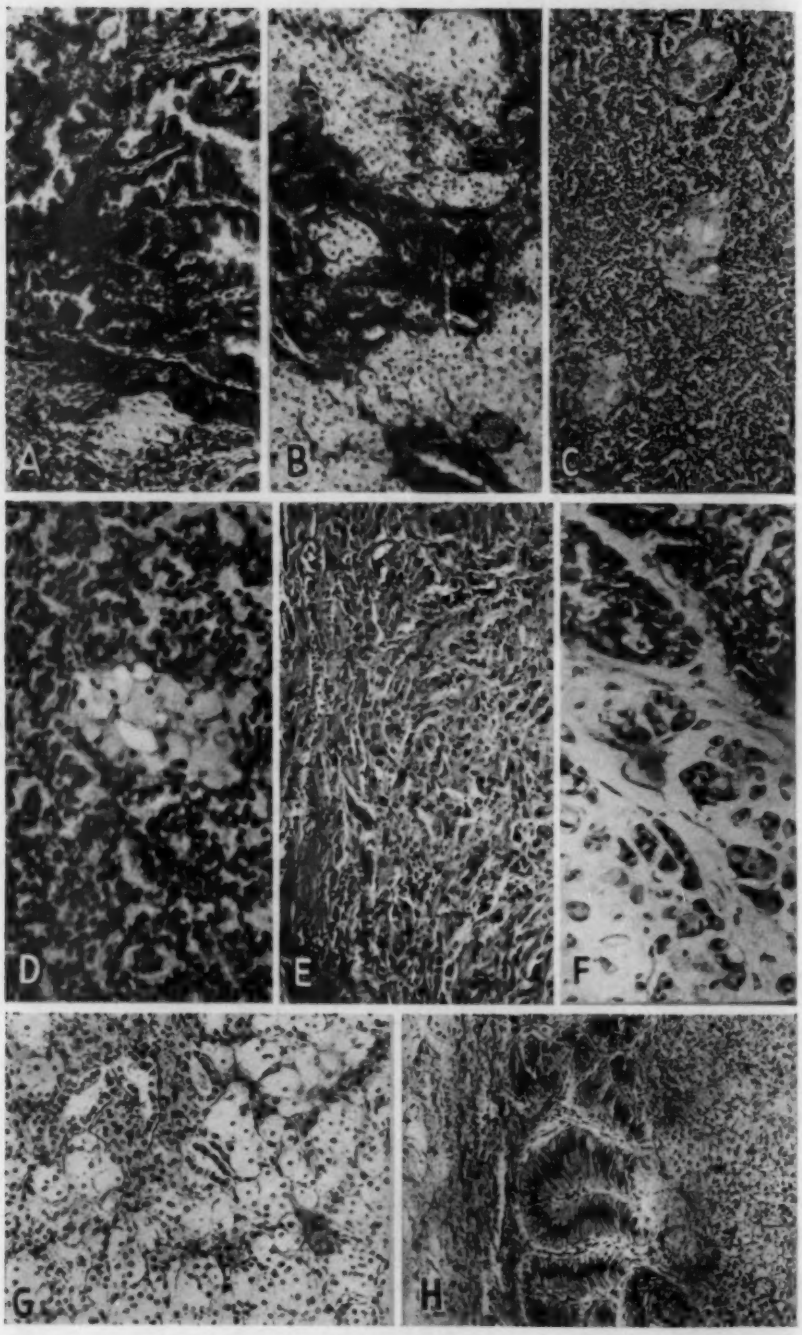

Figure 6

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for the initial as well as for the later phases. Neither in the large tumors nor in the metastases in general could I find typical adrenal elements as long as the cells were fixed to a wall, bordered a lumen or retained polar orientation (fig. 6 *A, B, C, D* and *E*). Evidently polarity is not compatible with adrenal character; in other words, adrenal character can be developed only after loss of polarity. Changes in cellular character due to loss of polarity can frequently be found in tumors. In the Krukenberg tumor of the ovary, for example, the carcinoma cells, as I have demonstrated, become signet ring cells merely when they float in the apolar mucin or when they invade the apolar stroma, thus losing their own polarity (fig. 6 *F*). Only in exceptionally rare cases do the tumors in the renal cortex develop from the beginning as adrenal cell nodes. In these rare cases no lumen, no papillary structure can be seen, but a solid apolar mass of large polyhedral cells with the typical tendency to store lipoid droplets in the cytoplasm is found. These cases are to be classified as instances of real primary "hypernephroma" of

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EXPLANATION OF FIGURE 6.

Renal cortical papillary adenoma of the right kidney in a case in which a malignant Grawitz tumor was present in the lower pole of the left kidney.

A, simple papillary structure with one row of dark-staining low cuboidal epithelium.

B, solid nodes formed by piling up of cells which fill the cavities and at the same time change into cells of the type forming the adrenal cortex. (The patient was a 50 year old white man.)

C, benign papillary adenoma of the kidney with several solid clusters of large polyhedral cells in the center of the type found in the adrenal cortex. (The patient was a 53 year old white man with a necrotizing Grawitz tumor the size of a walnut in the lower pole of the left kidney and deposits of the same tumor in the myocardium, the liver, the adrenals, the sacrum and the lumbar [first, second, fifth] and thoracic [twelfth] vertebrae; both kidneys presented numerous nodes varying in size from that of a pinhead to that of a cherry. On microscopic examination, the larger nodes were found to be deposits of the Grawitz tumor and the smaller ones well demarcated benign papillary adenomas.)

D, group of adrenal cells under higher magnification.

E, deposit of the Grawitz tumor in the myocardium. The tumor cells are elongated and similar to the elements of a spindle cell sarcoma.

F, so-called Krukenberg tumor, an ovarian deposit of carcinoma of the stomach. Note in the upper third of *F* carcinoma cells forming strands, with preserved polarity, and below, carcinoma cells which have lost their polarity and have become transformed into signet ring cells floating in a mucinous secretion.

G, renal cortical yellow node, 5 mm. in diameter, primarily solid, consisting of cells of adrenal cortical character (patient a 6 year old white girl with otitis media and purulent meningitis). This illustration is shown by courtesy of Dr. Otto Saphir.

H, adrenal cortex of a dog. Note the subcapsular loops formed by cells with restricted polarity. The middle zone is composed of polyhedral cells without polarity.

the kidney, without renal cells or structures. Such tumors occur but doubtlessly are rare and form only a small part of the great variety of tumors classified as Grawitz tumors (fig. 6*G*).

The observation that cells resembling those of the adrenal cortex develop from tumor tissue of renal character is not compatible with the conception of simultaneous misplacement of fetal cells of both tissues as offered by the Gerlachs. It leads to the assumption that the tendency for differentiation in the adrenal direction is hidden in the renal tissue as a latent potency or, as embryologists call it, a prospective potency. Such prospective potencies are physiologically present in great numbers and varieties at the beginning of fetal development and gradually decrease during intrauterine life. Till now there have been no means of determining the prospective potentialities of a cell by morphologic methods; one can make conclusions about their presence only by the differentiation observed under special conditions. The decision as to which of the initial potencies will be extinguished and which will survive in normal fetal life apparently depends on normal tissue relations. For most of the tissues, there is some other tissue which by its contact determines and controls the direction of differentiation. This determining tissue was called by Spemann the organizer. Prior to him Vera Dantschakoff demonstrated that for the gonads the genital cells function as organizers, and for the mutual relations between the müllerian duct, the wolffian duct and the gonad Gruenwald has published some very illuminating experiments. For the kidney the bud of the ureter is the organizer, which evokes tubular differentiation of the metanephric mesenchyma. If the bud of the ureter fails to reach the prospective renal tissue, no kidney develops. The ureter thus is the organizer for the kidney; the organizer of the adrenal gland is not yet known. It is known that the potency to form elements of the adrenal cortex is widely spread along the urogenital fold, as the accessory adrenal bodies prove. A part of the urogenital parenchyma, the metanephrogenic tissue, shares this potency, which is extinguished or at least not activated when the bud of the ureter organizes the production of renal tubular tissue. But this process of differentiation may not be thorough and complete and one or more cells may be left which still retain adrenal potentialities. These potencies may be transferred during mitotic and during amitotic division to coming cell generations like recessive characters and may suddenly become manifest. There is no way to see these potentialities under the microscope, and they may be hidden in cells which look perfectly normal. For pathologic prospective potencies there are good examples to corroborate this assumption. Tsutsui and Itchikava succeeded in producing carcinoma of the skin of mice by painting the skin with tar for several weeks. Lipschütz found that this holds not alone for a special area of the skin

but for each single field tested, back and front, ears and tail, extremities and head, all of which respond similarly by carcinogenesis if properly stimulated by prolonged application of tar. Since cutaneous carcinoma develops from malignant transformation or degeneration of the basal cells, one has to conclude that the basal cells anywhere in the skin possess the prospective latent property to respond by carcinogenesis if adequately stimulated. Neither Cohnheim's theory of local embryonic remnants nor Fischer-Wasels' local carcinoma germ harmonize with these experimental findings, but only the assumption that all normal basal cells of the mouse's skin possess the prospective latent potency to change into carcinoma cells. A similar change in the field of human pathology may be mentioned: In early phases of fetal life the whole celomatous endothelium probably possesses the prospective potentiality to develop into serous and ciliated, or into endometrial, or into high columnar mucinous epithelium. Normally these qualities are extinguished or at least not activated everywhere in the peritoneal cavity except in the fold which later forms the müllerian duct. This duct in its three parts differentiates into the mucosa of the fallopian tube, the endometrium and the mucosa of the cervix. If, as Heim pointed out, the endometrial potency which has been preserved in some area of the peritoneal endothelium is in later years evoked, it may give origin to an endometrioma. If, on the other hand, one of the three potentialities remains alive in the celomatous cells on the ovarian surface, which become dislocated as Walthard inclusion cysts in the albuginea, the waking up of these dormant potentialities years later may produce serous, endometriomatous or pseudomucinous cystomas. Similarly, adrenal prospective potentiality may remain in cells of the metanephric blastema. These cells may be incorporated in normal tubules without giving any microscopic evidence of their latent qualities, and these qualities may remain silent for years.[38] In a few exceptional and rare cases such cells may develop directly into primary adrenal nodules in the renal cortex. In general, the adrenal potencies of the cells become evident only after preceding development into cysts and adenomas of renal character; this is similar to the cervical potentialities of the ovarian surface cells, which do not become manifest as long as the cells lie at the surface but give origin to pseudomucinous cystomas after the cells have been displaced or dislocated as inclusion cysts into the albuginea. In the kidney, in general, first a tumor of renal character develops, and secondarily the adrenal potentiality becomes manifest. The latter phase is connected with the loss of polarity. Sometimes the two phases

38. The prospective potencies of mature, fully differentiated normal cells have been underestimated until the last few years. Gruenwald, in his discussion of the relations between the müllerian and the wolffian duct (Proc. Inst. Med. Chicago **13**:382, 1941) said: "Normal tissues have the potencies which the creators of the embryonic tumor theory believe to be present only in embryonic cells."

overlap to a certain extent; an adrenal characteristic, for instance fat or lipoid storage, may occur at a time when the tissue still presents the structure of a renal papilloma. Thus, the frequently seen mongrel or hybrid types of hypernephroma originate with renal papillomatous structure and lumen formation, built by cells with adrenal fat or lipoid storage in the cytoplasm—hybrid types which can never be explained by a mixture of independent renal and adrenal germs. Notwithstanding the fact that during fetal and extrauterine life the cells of the adrenal cortex of the human subject have no polar orientation, there may be a phylogenetic latent tendency for limited polarity. In some mammals—for instance, in the dog—the cells of the subcapsular loops of the glomeruli are spindle shaped with an elongated longitudinal axis (fig. 6*H*). It is possible that in the human subject also adrenal cortical cell character is compatible with limited polarity, at least during neoplastic transformation.

Whereas the adrenal prospective potency may be preserved and may survive even through the phase of renal tumor development, the quality to produce hormones which control, protect and elicit the characteristics of the opposite sex is lost. Hypernephromas of the adrenal gland and of the ovary frequently have been observed to virilize women; hypernephromas of the adrenal gland in a few cases have effeminized men, but such sex changes have never been observed with a Grawitz tumor or hypernephroma of the kidney. Evidently, this dyshormonic potency gets lost when the cells live and develop under the environmental influence of renal parenchyma.

SUMMARY

When Grawitz in 1883 traced the well known cystic-papillary and medullary carcinoma of the kidney to adrenal cortical elements, he based his histogenic explanation on the appearance of the large polyhedral cells and their pronounced tendency to store fat in the cystoplasm, as well as on the thin septums with thin-walled capillaries. Stoerk, who in 1908 rejected the theory and classified the questionable tumors as nephrogenic, founded his explanation on the observation that the tumors developed directly from the well known adenomas of the renal cortex, which originate from tubular epithelium and occur with particular high incidence in sclerotic kidneys. As arguments against adrenal origin he pointed to the fact that adrenal cortex has neither the potency to form lumens nor the tendency in normal or neoplastic proliferation to form papillary projections. Until now the argument has not been definitely settled. Some authors still support the renal, others the adrenal, character and origin of the Grawitz tumor. A few like Wilson and Willis and the Gerlachs accept both theories and trace the neoplastic tissue of renal character back to remaining embryonic renal cells and the adrenal

tissue to misplaced adrenal cells. Better than the simple cell character, which changes rapidly during the neoplastic development, the so-called functional structures, i. e., the physiologic and pathologic morphologic changes which occur in physiologic or pathologic functions, can be utilized to determine the type of cells with which one is dealing.

Since the time when nephrosis as a pathologic entity was separated from nephritis, the storage of abundant quantities of hyaline droplets in the tubular epithelium has attracted a great deal of attention, for this change is directly related to the characteristic excessive albuminuria. It is not specific for nephrosis, since it can be found frequently in renal amyloidosis and in the borderline zone of anemic infarcts. The hyaline droplets were traced back to local cellular degeneration, or to secretion from the blood, or to reabsorption from the glomerular filtrate in the lumen. Animal experiments, particularly those on amphibia and reptiles, proved that the droplets develop only when albuminous fluid is present in the tubular lumen. These characteristic hyaline droplets can also be found in the epithelium of Grawitz tumors when the cells line cavities and the cavities contain proteinic fluid. This proves the renal tubule character of the tumor cells and at the same time supports the origin of the hyaline droplets by reabsorption in nephrotic and amyloidotic kidneys. Another protein, eventually found in the epithelium of renal tubules, the hemoglobin, has been proved by similar experiments on reptiles and amphibia to be of glomerular origin, the result of absorption from the glomerular filtrate. Thus, in hematuria hemoglobin is found in the tubules as a result of passage of a bloody filtrate from the glomerulus into the tubular lumen. Frequently in Grawitz tumors the neoplastic cells lining cavities can be found loaded with hemoglobin or with hemosiderin when the lumen contains blood. This finding similarly speaks for the tubular character of the tumor cells and supports the theory of tubular reabsorption in the production of hematuria. Thus far these "functional structures" help to establish the renal character of Grawitz tumors. On the other hand, there cannot be any doubt that in some of the tumors massive parenchyma can be found whose polyhedral fat-storing cells perfectly duplicate normal or eventually neoplastic cells of the adrenal cortex. Observations on small initial cortical papillary adenomas demonstrate that adrenal tissue apparently originates secondarily, as a result of loss of cellular polarity, in a tumor of renal tubular origin. The proliferating cells form solid homogeneous islands, the elements of which assume cortical character. Embryologic considerations, the widely separated origins of the metanephros and the adrenal cortex, as well as the numerous renal cortical adenomas of adrenal cell character found far from the upper pole of the kidney, speak against the old theory of adrenal cells mechanically misplaced in the field of the kidney as the origin of

the Grawitz tumors. The histologic morphologic findings tend to support the modern embryologic conception that in early fetal times, when the mesenchyma is still pluripotent, the matrix of the renal and of the adrenal cortex possesses the potency to differentiate into both renal and adrenal tissue and that the heterotopic potencies are extinguished during fetal development. If latent adrenal cortical potencies remain in the renal parenchyma cells, such cells years later may give origin to adrenal tissue. This awaking of latent potencies occurs frequently during neoplastic proliferation of renal character. The presence of renal and adrenal potencies in the same cells may produce tissues of a hybrid type, i. e., cells with combined renal and adrenal characteristics. This can serve as an explanation for Grawitz tumors with cystic and papillary structures corresponding to tumors originating from renal cortical tubules but containing polyhedral fat-storing cells resembling cells of the adrenal cortex.

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A SIMPLER CLASSIFICATION OF MAMMARY TUMORS

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Having classified a group of tumors, one may believe that one has thereby solved the problems of their etiology and treatment. Obviously this is a mistaken notion. Classification may and should lead, however, to clarification. When it comes to tumors of the breast, the pathologist, the surgeon and the teacher alike find themselves bogged down in a miscellany of terms that are most confusing and call for rearrangement. It is the object of this paper to attempt a simpler classification and thus make the subject more homogeneous than it appears under the present nomenclature.

In a recent paper Boyd [1] stated that there are two groups of classifiers: the "lumpers" and the "splitters." He expressed the opinion that both have their function in the study of tumors. The classification proposed in this article will be based on the histology of the breast and will fall into the "lumped" rather than into the "split" category, although a little judicious splitting may occur here and there in order to accommodate the older and more familiar categories to the simpler needs of this paper. Such a classification must be one that will be readily understood by those who have hitherto depended on such terms as "scirrhous," "simplex," "medullary," "mucous" and the like, which are obviously applicable to variants of larger types. An attempt will be made to unite them in more comprehensive categories, explaining in the meantime why they merit this coagulative treatment.

Before proceeding with classification, it will be necessary to recapitulate briefly the embryology and the histology of the breast, as it will be on this framework that the classification will be erected.

ANATOMIC CONSIDERATIONS

It is well known that the breast is a modified sweat gland, that it develops through a downgrowth of massed cells from the epidermis into the pectoral region, cells that later begin to form branching coarse tubules that ultimately constitute an inverted tree of larger and smaller

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1. Boyd, W.: Canad. J. M. Technol. **3**:51, 1941.

ducts and canaliculi. During lactation one finds acini grouped in grape-like clusters at the extremities of canaliculi. One may thus recognize large collecting ducts, medium-sized or lobular ducts and finally a multitude of canaliculi with terminal acini. The fact that the medium-sized ducts sometimes become acidophile, their cells staining red with eosin or fuchsin, while their cytoplasm becomes somewhat opaque, has caused some authors (Lee, Pack and Scharnagel [2]) to draw a parallel between the appearance of these ducts and that of the sweat glands, from which they deduce that these structures are true sweat glands intercalated among the lacteal ducts. This rather unappetizing assumption is nowhere borne out by histologists, and one may search in vain for any reference to such an intermingling of sweat and lacteal glands in histologic literature on the normal breast. It seems more probable that the medium-sized lacteal ducts have a tendency to take on this appearance under conditions that give rise to chronic fibrocystic disease, as they are most prominently and frequently observed in the Schimmelbusch type of that malady.

The question as to whether acini are developed only in lactating breasts and are absent in virgin mammary glands seems to remain debatable despite the observations of Rosenburg,[3] made in 1922. He published two articles, one of them [4] merely a warning to surgeons concerning certain physiologic changes in the mammary gland and the other a careful documentation of a study of 56 subjects at autopsy, in which the breasts were examined and the findings correlated with those in the ovaries, the menstrual phase of which had been determined by gross and microscopic examination. He found that during the intermenstrual period the mammary gland was in a condition of complete and primitive atrophy, resembling a male breast, whereas it became hyperplastic and showed development of acini shortly before menstruation set in. Not only did he find this phenomenon to prevail in nulliparae, but he was able to check it against observations in multiparae, whose mammary glands showed exactly the same pictures irrespective of the history of past pregnancies and lactation. His photomicrographs are highly illuminating.

These observations go a long way to explain the disputes which have arisen as to the presence or the absence of acini in the normal mammary gland. Premenstrual hypertrophy should, for this reason, be carefully

2. Lee, B. J.; Pack, G. T., and Scharnagel, I.: Surg., Gynec. & Obst **54**: 975, 1933.

3. Rosenburg, A.: Frankfurt. Ztschr. f. Path. **27**:466, 1922.

4. Rosenburg, A.: Zentralbl. f. Chir. **50**:510, 1923.

considered by surgeons before a "lumpy breast" is explored by operation. It must be admitted that surgical pathologists seldom see the atrophic phase of the mammary gland, as the breast in this phase would not come under the knife. Rosenburg's article, however, provides stimulating material for a simple form of statistical research and confirmation of his findings.

In order to make a classification of tumors of the breast on an anatomic basis one needs but to break down that organ into its components and fit the neoplasms to the appropriate tissues. There is one class of tumors in which fibrous tissue appears to play an almost equally important role with epithelium and which, for that reason, must be assigned to the epithelial group somewhat illogically.

ANATOMIC CLASSIFICATION

Tumors arising in the skin (papilloma, cutaneous cancer, melanoma, and others) need not be discussed at length; they should be classified as tumors of the skin, not as mammary growths.

With respect to the nipple, three types of tumor might be met with: one within the areola, one within the large ducts and about their openings, and the third in the smooth muscle about these. The last may be dismissed as hypothetic, a type of leiomyoma that one seldom if ever encounters. The first type is spiradenoma. It may develop in the apocrine sweat glands of Montgomery and sometimes produces cancer, true sweat gland carcinoma of the areola. The second is Paget's disease of the nipple, in which the epidermis shows not only the changes of chronic eczema but in its basal layer the development of characteristic cells, which are known as Paget's cells. Concomitant with this change, there is overgrowth of the lining cells of the large lacteal ducts and the production of duct cell carcinoma. Thus Paget's disease is a cancerous condition at the junction of the mucosa and the large ducts of the nipple, affecting both elements alike.

To classify tumors of the mammary gland proper as simply as possible, one might divide them into (*a*) duct cell, (*b*) canalicular and (*c*) acinous. As the acini are possibly rather transitory structures, it is natural that neoplasms derived from them should occur very rarely; conversely, as the canaliculi are by far the most numerous elements of the breast, it is equally natural that most of the epithelial mammary tumors should originate in them. To this simple classification, a qualifying subdivision should be added: noncancerous and cancerous groups. One epithelial tumor must elude this classification on account of its tendency to be composed of an exudate-like mass of discrete cancer

cells, the origin of which may not be accurately determined. This is the so-called "acute" carcinoma most often seen in lactating breasts. Here the growth is so atypical that it would be presumptuous to identify any definite point of origin. It might be well to remark that although I propose to classify mammary tumors according to the regions they most resemble, this resemblance may be merely a matter of cell grouping and tumor growth and might not necessarily indicate that the tumor arose from, say, canaliculi or acini. It seems very likely, however, that tumors resembling canaliculi arise from canalicular epithelium and so on. Following out this scheme of classification, then, one might group the tumors of the mammary gland proper as follows:

Duct Cell Tumors
- Noncancerous group
  - (*a*) Simple adenoma (including "comedoadenoma")
  - (*b*) Papillary adenoma ("intraduct papilloma")
  - (*c*) Papillary cystoma
- Cancerous group
  - (*a*) Simple duct cell carcinoma
    1. Solid type ("comedocarcinoma")
    2. Tubular type
    3. Epidermoid type
  - (*b*) Papillary duct cell carcinoma (papillary adenoma that has undergone carcinomatous change)

Canalicular Tumors
- Noncancerous group
  - (*a*) Simple or glandular adenoma
  - (*b*) Fetal adenoma
  - (*c*) Fibroadenoma (intracanalicular and pericanalicular fibroadenoma)
- Cancerous group
  - (*a*) Pure epithelial canalicular carcinoma
    1. Simple type ("carcinoma simplex")
    2. Alveolar type ("medullary carcinoma")
    3. Adenomatoid type ("adenocarcinoma")
    4. Mucous type ("colloid carcinoma")
    5. Scirrhous type
    6. Acute type ("diffuse carcinoma")
    7. Carcinoma in situ
  - (*b*) Mixed tissue cancer
    1. Intracanalicular sarcoma
    2. Canalicular fibroadenocarcinoma

Acinous Tumors
- Noncancerous acinous adenoma
- Acinous carcinoma ("solid carcinoma")

TUMORS OF THE MAMMARY GLAND PROPER

DUCT CELL TUMORS

Noncancerous Group.

Simple Adenoma.—Simple adenoma is sometimes seen in the large ducts; it consists either of solid masses of rather large indifferent cells or of a "honeycomb" of smaller channels into ductlike or acinoid spaces that may become cystic. It is also noted in connection with fibrocystic disease and with duct cell carcinoma. The more solid form may resemble what Bloodgood called "comedoadenoma," the noncancerous form of his "comedocarcinoma," so called because wormlike casts of detritus may be squeezed from the parenchyma of the tumor, just as they may be expressed from comedos or "black heads" in the skin of adolescents.

Papillary Adenoma (Intraduct Papilloma).—This is the very common, usually small, readily bleeding tumor of the ducts of the deeper portions of the nipple. It differs from simple adenoma only so far as it tends to push out papillary projections into the duct lumens. Both types, the simple and the papillary, may be seen in the same microscopic fields. This makes sharp distinctions rather pedantic.

Papillary Cystoma.—There is a papillary tumor that arises in those larger and intermediate ducts that have undergone the acidophilic and cloudy changes already referred to. It resembles spiradenoma rather noticeably and differs from the intraduct papilloma just noted in that the papillae which it forms do not contain subsidiary lumens or gland-like structures but are solid and rather opaque and acidophilic, growing into the lumens in cauliflower-like excrescences. Their beginnings are often noted in the Schimmelbusch type of chronic fibrocystic disease of the breast, in which many incipient papillomas may be seen. They may exhibit a very well developed outer layer of myoepithelium or even straplike smooth muscle cells, which heighten the resemblance to sweat ducts. This tumor seems seldom to undergo cancerous change, although occasionally carcinoma has something of the appearance of papillary cystoma plus cancerous characteristics and might therefore develop from it. Such growths are often called "sweat gland carcinoma of the breast."

Cancerous Group.

Simple Duct Cell Carcinoma.—(*a*) Solid type, or "comedocarcinoma." This type forms solid plugs or heavy tubes about necrotic centers in the larger ducts. It may be limited to the ducts, occurring in situ (Foote and Stewart [5]), or it may break out into the stroma, whereupon it takes on the characteristics of scirrhous carcinoma, which will be discussed later. A duct cell carcinoma of this type that fails to show this breakout (or concomitant and independently developing scirrhous

5. Foote, F. W., Jr., and Stewart, F. W.: Am. J. Path. **17**:491, 1941.

carcinoma) usually bears a good prognosis and fails to metastasize. It is apt to be rather heterogeneous, however, and one must not be misled by finding several blocks of tissue that look comparatively quiescent, for one may have missed an area in another block that would have shown more malignant characteristics. For this reason, one usually treats these tumors by radical surgical removal.

(*b*) Tubular type. Sometimes one sees a tumor in the somewhat smaller subdivisions of the larger ducts that shows a good deal of metaplasia, does not grow in plugs but tends to take the form of cords or tubes of metaplastic cells. It seems to arise from the larger ducts and suggests a subdivision of duct cell carcinoma.

(*c*) Epidermoid type. This is rarely seen. It is a true epidermoid or squamous cell carcinoma that grows in the larger ducts, destroys them and escapes into the stroma. Its cells are mostly of the spinous or prickle cell variety rather than truly squamous and keratinized, although keratinized pearls may be formed in abundance. It appears to be very malignant and metastasizes early and widely. Such a growth shows no continuity with the overlying epidermis, seemingly arising by a process of metaplasia of the duct epithelium similar to that which is observed in epidermoid carcinoma of the gallbladder, urinary bladder, renal pelvis and the like (Foot and Moore [6]).

Papillary Duct Cell Carcinoma.—Papillary adenoma sometimes undergo cancerous transformation to form papillary cystadenocarcinoma or "adenoma malignum." This often creates vexatious problems as to which surgical course should be taken, as some neoplasms of this type may show very little cancerous deviation from papillary adenoma. In our experience, simple excision of these more innocent-looking growths has usually proved to be insufficient and has been followed by recurrence in a more malignant form.

CANALICULAR TUMORS

Noncancerous Group.

Simple or Glandular Adenoma.—This often closely resembles accessory mammary tissue, so well is it differentiated. Tumors of this type vary from small globular ones characterized by branching ducts that taper to bulbous acini to larger growths that take on a more complex appearance, the canaliculi becoming thicker walled, with more primitive cells and a less definitely canalicular organization. The latter may show many cystic dilatations and in some instances produce very bizarre structures. Both types are well encapsulated and nonmalignant.

6. Foot, N. C., and Moore, S. W.: Am. J. Cancer **34**:226, 1938.

Fetal Adenoma.—This name was applied by Ewing [7] to a growth resembling the adenoma just described, except that it is composed of innumerable canaliculi, shows no acinous structures and has a loose reticular stroma. The lining of the tubules is low cuboidal epithelium resembling that of the fetal mammary gland. Tumors of this type may show active proliferation of both the epithelial and the connective tissue elements, with many mitotic figures present, yet they are not clinically cancerous, and after removal, which is facilitated by their firm and definite capsule, they do not recur.

Fibroadenoma.—Two forms used to be recognized, but as they may be seen in the same tumor at the same time, this distinction appears to be useless. Cheatle and Cutler [8] described three types: (1) subepithelial, in which the fibrous growth occurs within the elastica of the canaliculus and does not distort this structure; (2) pericanalicular and periacinous, in which the growth lies outside of the elastica and, if it invades the lumen, pushes this ahead of itself, distorting the luminal outline, and (3) a mixed one combining the two preceding types. The presence of the mixed type in the classification would seem to nullify the necessity for the other two. Thus it seems best to drop the terms "pericanalicular" and "intracanalicular" adenofibroma and call such neoplasms simply fibroadenoma. Fibroadenoma resembles adenoma of the types just considered except for the large amount of fibrous tissue that it contains, which often exceeds the glandular elements in quantity. This may surround canaliculi in thick collars; it may grow into their lumens in the form of broad, stumpy plugs, over which the epithelium is spread out into a thin sheet. Sometimes there is an increase in the epithelium until it forms subsidiary papillary projections on the surface of the larger fibrous papillae. All this results in bizarre geographic patterns in microscopic sections, which are quite characteristic. In tumors of this type the fibrous elements may undergo myxomatous degeneration. This occasioned the coining of the term "adenomyxofibroma," which is descriptive but clumsy. Whether the changes that produce these tumors reside in the epithelial tissue or in the stroma is a much disputed question. As malignant tumors may be seen with carcinomatous characteristics, while others show sarcomatous stroma and innocent epithelium, one may not seek an answer there. That they should be grouped with adenoma is the usually accepted conclusion, and Saphir [9] has recently proposed a classification not unlike this one.

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7. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940.

8. Cheatle, G. L., and Cutler, M.: Tumours of the Breast, Philadelphia, J. B. Lippincott Company, 1931.

9. Saphir, O.: Am. J. Path. **15**:605, 1939.

Cancerous Group.

Pure Epithelial Canalicular Carcinoma.—The tumors thus designated seem to be of canalicular origin or, at least, to simulate canaliculi in their growth habitus. They present various types of growth under varying conditions; sometimes several types may be seen in the same tumor, and it is this that makes one hesitate to continue to use the older nomenclature. The types presented in that nomenclature may, however, be continued as subvarieties of canalicular carcinoma in this newer scheme, as they occasionally afford the surgeon clues as to prognosis and treatment.

(*a*) Simple Type: This is the former "carcinoma simplex," a tumor the cells of which are rather opaque and spherical to cuboidal and tend to grow in cords or elongated masses without forming ductlike structures.

(*b*) Alveolar Type: This was known as "medullary," less frequently as "alveolar," carcinoma. The first term implies that the tumor presents a marrow-like appearance on gross inspection, but the acinous carcinoma (to be described later) is more marrow-like than this one, which is usually firm and unlike marrow. The term is a poor one. The word "alveolar" may be properly retained, as the cells tend to be grouped in alveolar spaces.

(*c*) Adenomatoid Type: A tumor sufficiently well differentiated to form definite ducts and to hint at structures resembling acini was called "adenocarcinoma." As this tumor is composed almost entirely of abortive canaliculi with occasional cystic dilatations, it is better to call it adenomatoid than to call it adenomatous.

(*d*) Mucous Type: This was known as "colloid carcinoma" and latterly as "mucous" or "mucinous adenocarcinoma." Often there is so much mucus secreted that the cells undergo degeneration and float about in masses of mucus. It is not always easy to decide whether the mucus is secreted by the cells or results from their degeneration. The tumor is admittedly less malignant than other forms of canalicular carcinoma, but its appearance of degeneration is deceptive, and if some of these cells metastasize to lymph nodes, they will grow there in the alveolar form and show much less, if any, mucus. The presence of this substance in carcinoma of the breast (as demonstrated by mucicarmine) is said by some pathologists to augur well for the prognosis. In some cases the more malignant-looking canalicular carcinoma may show a good deal of mucus when stained with this form of carmine and is then said to carry a better prognosis (Frantz [10]).

10. Frantz, V. K.: Am. J. Cancer **33**:167, 1938.

(*e*) Scirrhous Type: In the majority of instances canalicular carcinoma provokes a very lively overgrowth of connective tissue about its cells, often to such an extent that the new tissue seems to throttle these elements, which are small and dense and grow in chains or cords. Such tumors are almost 90 per cent ligneous connective tissue and 10 per cent cellular material. Let such cells, however, penetrate to the axillary lymph nodes and they will form alveolar masses that provoke little fibrous response in most instances, although they may occasionally carry their desmoplastic propensities with them. Scirrhous carcinoma is not to be lightly dismissed just because of this desmoplasia, or fiber-provoking trend. The fibrous tissue may appear to wall the cancer off, but it is common experience that the walling off is more apparent than real.

(*f*) Acute Type: This form probably arises from the canaliculi, although similar tumors may be observed around the larger ducts. In this tumor the cells grow independently of one another and resemble an exudate of discrete tumor cells, rather than a tumor.

(*g*) "In Situ" Type: Canalicular carcinoma in situ is a form recently described and discussed by Foote and Stewart.[5] In this the cells do not leave their situs in the canaliculi but undergo metaplastic changes while still a part of the lining of those structures. This condition is often seen in connection with the Schimmelbusch type of chronic fibrocystic disease of the breast. The authors have made a valuable contribution in calling it to general attention and stressing its potentially dangerous character. If left alone, the neoplasm will almost inevitably break away from the canaliculi and produce invasive canalicular growths. The tumor is often diagnosed as "areas of metaplasia in Schimmelbusch's disease," but it has been recognized that the prognosis does not differ with the terms used in the diagnosis.

Mixed Tissue Cancer.—(*a*) Intracanalicular Sarcoma: One occasionally observes a papillary intracanalicular fibroadenosarcoma, or "sarcoma phylloides cysticum," in which the accent of growth is on the stroma rather than on the epithelium. This tumor grows to enormous size, rarely metastasizes and is deceptive in its microscopic appearance, which resembles that of fibrosarcoma rather closely. The epithelium is spread out into a thin membrane that is tensely extended over enormous masses or knobs of embryonal or metaplastic connective tissue. As the tumor bears an undeniable resemblance to the intracanalicular form of canalicular fibroadenoma, one must conclude that it is derived from that tumor, and indeed it differs from that tumor chiefly in degree.

(*b*) Canalicular Fibroadenocarcinoma: Sometimes canalicular fibroadenoma will show cancerous changes in the epithelial elements suffi-

cient to warrant classifying it with carcinoma, but one should always be wary before making such a diagnosis as noncancerous fibroadenoma may show considerable epithelial hyperplasia and even some metaplasia without being really malignant.

ACINOUS TUMORS

These tumors are decidedly rare and said to be rather well behaved. As has been indicated, it is always difficult to ascribe an acinous origin to a mammary tumor so long as any doubt exists as to the presence of acini in the resting mammary gland. Rosenburg's work indicates that there is a possibility of such a tumor being formed during the period of premenstrual mammary excitation.

Noncancerous Adenoma. — Acinous adenoma is much like the glandular canalicular adenoma of the preceding section. It is a matter of mere academic interest to attempt to distinguish them by the predominance of the acinous or the canalicular architecture in a given tumor.

Acinous Carcinoma.—This is usually a rather bulky growth that spreads more slowly than the types already enumerated, although it may grow rapidly within its capsule and attain considerable size. It may have a brownish or reddish color rather than the pearly white of canalicular carcinoma and is so predominatingly epithelial as to be rather soft and friable on palpation. It does have the appearance of bone marrow in many instances and may have occasioned the name "medullary carcinoma" which has already been alluded to. Microscopically, the epithelium resembles that of the acini and may differentiate into a double layer about a lumen. This resemblance may be fortuitous and misleading, but it has been noted. The cells may be small and vacuolated, like secreting acinous cells, or they may be pleomorphic (with many tumor giant cells among them) and not suggest an acinous origin. The growth tends to metastasize late and to bear a relatively good prognosis after operation. That it is distinctly rare may be explained on the basis of the fleeting existence of the much discussed acini. At all events, this massive tumor (sometimes called solid carcinoma) is a distinct, if not always readily recognized, entity. It is placed in the category of acinous carcinoma in this scheme of classification because it seems to fit into that category theoretically.

CONNECTIVE TISSUE TUMORS

Fibrous Type.—The collagenous tissue of the breast can be the site of hard or soft fibroma, though these are rather rare. Fibrosarcoma represents their cancerous analogue.

Reticular Type.—The reticuloendothelium that forms the sheaths of the canaliculi and ducts, creating a sort of expansile space between these and the denser collagen of the breast, may give rise to reticulum cell sarcoma. Apparently it is not generally understood that this packing of loose reticular tissue exists about the tree of the mammary gland. The tumor metastasizes very early to other depots of reticular tissue, such as the axillary lymph nodes, so that it is sometimes difficult to decide whether it is primary in the breast and not metastatic from lymphoid tissue. A full discussion of the subject will be found in a monograph by Puente Duany,[11] who has collected a number of cases and reviewed the literature carefully. He considers them to be a form of lymphosarcoma, however, which strikes me as rather confusing.

Mucoid Type.—Pure myxoma of the breast is seldom seen, but myxomatous degeneration of the canalicular fibroadenoma is common. Myxoma and myxosarcoma are occasionally encountered.

Adipose Type.—Lipoma and very rarely liposarcoma of the breast occur. The former is rather unremarkable and it is often hard to convince one's self that the tumor is true lipoma rather than a focus of redundant fatty tissue.

Osseous and Cartilaginous Type.—A tumor of aberrant bone or cartilage is sometimes found in the breast and probably represents choristoma, a tumor of displaced fetal tissue. It may or may not be cancerous.

TUMORS ORIGINATING IN OTHER TISSUES IN THE BREAST

Muscle.—The hypothetic occurrence of smooth muscle tumors about the nipple has already been discussed. Occasionally a tumor of the skeletal muscle of the breast is recorded, and usually it is rhabdomyosarcoma.

Nerve Tissue.—Tumors of peripheral nerves, such as neurogenous fibroma, neurilemmoma, ganglioneuroma and neurosarcoma, may occur here as elsewhere but are not peculiar to the breast.

Vascular Tissue.—Growths may originate in the blood and lymph vessels of the breast; they need no special consideration.

Lymphoid Tissue.—There is very little lymphoid tissue in the breast itself, and most of it is in the form of extensions from axillary nodes into the "tail" of the organ. While Puente Duany's monograph is entitled "Lymphosarcoma of the Mammary Glands," the primary tumors described by him are almost entirely reticuloendothelial rather than lymphoblastic.

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11. Puente Duany, N.: Linfosarcoma de las glándulas mamarias, Habana, Cárdenas y Compañia, 1941.

ADDITIONAL CONSIDERATIONS

Secondary Tumors of the Mammary Gland.—Secondary tumors of the breast are rarely seen and are metastatic. Hence they do not affect this classification and are included here only as unusual possibilities.

Ectopic Mammary Tissue.—Frequently one finds small mammary glands in the axillas; more rarely, along the fetal "milk line." These glands may be the site of any of the tumors to which the breast is heir. We have encountered several tumors of these glands at the New York Hospital within the past decade. One was a canalicular tumor, diagnosed as fibroadenoma, which reached a diameter of about 10 cm., in a girl of 13.

RECAPITULATION

Tumors of the breast can be readily classified according to a system based purely on their histogenetic characteristics. It is particularly recommended that epithelial tumors of the breast be arrayed in three groups only; duct cell, canalicular and acinous. It is also to be noted that the last group is somewhat hypothetic and that there are those who do not believe in its existence. The classification could then be further simplified by omitting this group and reallocating the two members.

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SECONDARY CANCER OF THE SPLEEN

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Secondary cancer of the spleen is relatively uncommon. Most authors give the frequency as ranging from 0.3 to 4.8 per cent of the total number of persons with cancer on whom necropsy is done (Krumbhaar [1]; Warren and Davis [2]; Guttman [3]). It is because of this infrequency that many investigators have directed their attention to the spleen in an effort to determine the reasons for the paucity of involvement. These resolve themselves into two main groups: first, the mechanistic and, second, those which have in view some inherent or acquired antipathy of the spleen toward neoplastic growths.

The mechanistic reasons appear to be the more plausible of the two and are supported by such observers as Kettle,[4] Wood [5] and Krumbhaar.[1] It is generally agreed that the white and red pulp of the spleen contain no lymphatic vessels, while the capsule and the thicker trabeculae contain only a few, and these are ill developed (Maximow and Bloom [6]). Carcinoma is by far the most frequent type of cancer, and although it metastasizes by the blood stream, it usually follows the lymph vessels. Therefore, the lack of lymphatics in the spleen at once precludes many cases of splenic involvement. The second peculiarity of the spleen is its constant change in size, which prevents anchorage and growth of many cancer cells that may have reached it through the blood stream. These splenic contractions were minimized by Dial,[7] who pointed out that the lungs, the contractions of which are more constant and severe, are a very frequent site of metastasis. Finally, the tortuosity and the acute angles of the splenic artery are held by Sappington [8] to prevent many cancer

From the Clinical Laboratories, Jefferson Medical College Hospital.

1. Krumbhaar, E. B.: Ann. Clin. Med. **5**:833, 1927.
2. Warren, S., and Davis, H.: Am. J. Cancer **21**:517, 1934.
3. Guttman, P. H.: California & West. Med. **52**:156, 1940.
4. Kettle, E. H.: J. Path. & Bact. **17**:40, 1912.
5. Wood, F. C.: Notes on Tumors, New York, J. T. Dougherty, 1920, p. 48.
6. Maximow, A. A., and Bloom, W.: A Text-Book of Histology, Philadelphia, W. B. Saunders Company, 1930, p. 400.
7. Dial, F.: Am. J. Path. **6**:79, 1930.
8. Sappington, S. W.: J. A. M. A. **78**:951, 1922.

emboli from reaching the spleen. This probably does not play a marked role, since emboli from cardiac vegetations with resulting splenic infarcts are common (Dial [7]; Warren and Davis [2]).

The antipathy of the spleen toward neoplastic growth has received a great deal of attention. Because the results are so contradictory, no conclusions can be drawn, and for the present the matter must be considered as unsettled. Woglom,[9] in his critical review on immunity to transplantable tumors, gave an excellent summary of the experimental work up to 1929. He divided the methods used into three groups: (1) those in which the tumor was transplanted directly into the spleen, (2) those in which tumor and spleen were mixed before inoculation and (3) those in which the tumors were studied in splenectomized animals. In each instance there was a difference of opinion as to the role played by the spleen. In general, however, it seems that those observers who found that the spleen does not have definite antineoplastic activity are more numerous than those who found that it has.

Summary of Cases

Primary Tumor	Cases	Number in Which Spleen Was Involved
Carcinoma		
Stomach	76	4
Bronchus	66	4
Pancreas	31	4
Breast	26	3
Bladder	28	2
Rectum	34	1
Ovary	17	1
Maxillary antrum	1	1
Melanoma	8	1
Lymphosarcoma	16	2

REPORT OF CASES

Incidence.—At the Jefferson Medical College Hospital in the last decade 640 persons with cancer came to necropsy. In 23, or 3.6 per cent, of these the cancer secondarily involved the spleen. Fifteen, or 2.4 per cent, of the splenic growths were due to metastasis, while 8, or 1.2 per cent, were due either to implantation from generalized abdominal seeding (2 cases) or to direct extension from neighboring organs (6 cases).

Primary Sites of the Splenic Neoplasms.—Practically all of the major organs of the body have been primary sites of neoplasms which have secondarily involved the spleen. The accompanying table is a summary of our cases. It shows the type and the primary site of the cancer, the total number of cases encountered and the frequency with which the spleen was involved.

Carcinoma of the stomach in each instance involved the spleen by direct extension. In 3 of the 4 cases of carcinoma of the pancreas

9. Woglom, W. H.: Cancer Rev. 4:129, 1929.

involving the spleen, the neoplasm was metastatic, while in 1 it reached the hilus of the spleen by extension. We have included 2 cases which we believe were instances of lymphosarcoma metastatic to the spleen, although we are, at the same time, fully aware of the fact that a multicentric origin cannot be absolutely excluded. In one case tremendously enlarged retroperitoneal nodes were considered as the primary site. The spleen weighed 1,000 Gm. and contained numerous circumscribed nodules measuring up to 2 cm. in diameter. In the other case there was a large ulcerating mass in the wall of the lower part of the ileum. The splenic pulp contained only two small, well circumscribed nodules, each measuring 3 mm. in diameter.

Metastasis to Other Organs.—Splenic metastasis is usually found in those cases in which there is generalized dissemination of the tumor. In the series reported by Warren and Davis,[2] each case with splenic metastasis showed also metastasis in three or more other organs. In 4 of our cases generalized metastasis was not shown. In one of these there was a bronchogenic carcinoma of the lower lobe of the right lung. The spleen was the only organ which showed secondary involvement. In another case a carcinoma involved the right maxillary antrum. It showed metastasis to one lung and to the spleen. In a third case there was a carcinoma of the pancreas. There were metastases to the regional lymph nodes, the liver and the spleen. The fourth case was one of lymphosarcoma of the lower part of the ileum with metastasis to the draining lymph nodes, both adrenals and the spleen. The remaining 19 cases showed widespread dissemination of the tumor.

Involvement of the Spleen.—Splenic involvement may be limited to the capsule or to the pulp, or it may include both the capsule and the pulp. The lesions limited to the capsule are the result either of direct extension from a neighboring tumor or of implantation from generalized abdominal seeding. In none of our cases was the capsule involved from a primary metastatic lesion. Lesions limited to the pulp alone are metastatic. In only a single case was there a combination of a tumor in the capsule and a tumor in the pulp. In this case a carcinoma of the stomach involved the hilus of the spleen by extension. The pulp contained several small nodules.

When the splenic involvement is recognized macroscopically, the tumors are usually nodular. They do not differ in any way from metastatic growths in other organs. Some are so small that they are barely visible. Others measure several centimeters in diameter. They are usually sharply circumscribed. The smaller ones are homogeneous and firm, while the larger masses may show areas of softening, necrosis or hemorrhage. Diffuse splenic metastasis is sometimes seen. This is rarely recognized grossly since the organ presents only a diffuse increase in firmness and is not necessarily enlarged (Kettle[4]). In 22 of our

cases the splenic growths were recognized macroscopically. All of these were nodular in type.

Microscopically, there is some difference of opinion as to what constitutes a metastatic lesion. Krumbhaar [1] stated that "cells in the blood stream do not properly constitute a metastatic growth." Warren and Davis,[2] on the other hand, expressed the belief that "any cluster of viable tumor cells separated from adjoining tissue should be considered as metastatic." Krumbhaar,[1] in his series of 40 cases, found only 3 in which the metastases were not recognized grossly. In approximately one half of the 46 cases reported by Warren and Davis [2] the metastases were recognized macroscopically, while in the other half such growths were recognized only microscopically. We found only a single case in which the metastasis was not recognized grossly. Small clusters of tumor cells, varying in number from a few to fifty or sixty, were found in the sinusoids. The primary lesion was an anaplastic carcinoma of the urinary bladder.

The nodular metastasis does not differ histologically from those in other organs. Several findings, however, are worthy of note. First, tumor cells in the spleen produce little or no necrosis of the surrounding splenic tissue. There is consequently extremely little peripheral fibrous tissue formation (Kettle [4]). This held true in all our cases. The only instances showing an increase of fibrous tissue around the tumor nodules were those in which the tumors themselves were desmoplastic. The cellular growths had no fibrous tissue delineation. This does not coincide with Krumbhaar's [1] views. He found fibrosis frequently and suggested that one reason for the paucity of splenic metastases may perhaps be the ability of the spleen to deal with tumor metastasis by a fibrosing process.

Second, the splenic veins of the trabeculae sometimes contain clusters and masses of tumor cells. This occurred in one of our cases. The patient had a carcinoma of the body of the pancreas. The spleen contained several metastatic nodules. Unfortunately, no mention is made in the protocol as to whether or not there was direct extension into the splenic vein. Thus, it cannot be stated whether the metastasis was retrograde by way of the splenic vein or whether the cells in the veins were a direct extension from the splenic nodules.

Third, of the 8 cases reported here in which there were capsular tumors from direct extension or from implantation, none showed involvement of the trabeculae and only one showed distant nodules in the splenic pulp. Thus, the paucity of capsular and trabecular lymphatics, together with the minor role which they undoubtedly play in metastatic lesions of the spleen, is given further support.

Splenic enlargement may or may not occur. In 1,000 cases of cancer Barron and Litman [10] found splenic enlargement seven times. Once the

10. Barron, M., and Litman, A. B.: Arch. Int. Med. **50**:240, 1932.

increase in size was due to splenic metastasis; once it was due to extension of an abscess from the colon, while in 5 cases it was caused by congestion from pressure of enlarged lymph nodes on the splenic vein. In the case reported by Sappington [8] the marked splenic enlargement (20 by 12 by 6 cm.) was due to diffuse metastasis from carcinoma of the breast. In another case of carcinoma of the breast, reported by Kraft,[11] the spleen weighed 2,000 Gm. It was diffusely infiltrated with tumor cells. The spleen in 8 of our cases weighed between 200 and 1,000 Gm. In 3 others the protocols read "two times the normal size," "very much enlarged" and "enlarged." Neither the weights nor the dimensions of the three spleens were recorded. In 5 of these 11 cases the enlargement was due entirely to splenic metastasis, while in the remaining 6 it was due both to tumor involvement and to congestion from pressure on the splenic vein. In 10 cases the spleen weighed less than 200 Gm. In the 2 remaining cases no mention was made of the size or the weight of the spleen.

Experimentally, Woglom [9] showed that there is no relation between splenic enlargement and resistance to tumor implants. He measured the spleens of mice before and after immunization and inoculation with tumor, and found no difference in the two measurements. Splenomegaly, nevertheless, is important clinically. Since it does occur in an appreciable number of cases of secondary neoplasm of the spleen, a possibility of such involvement should be considered in the differential diagnosis of any splenic enlargement.

SUMMARY

Twenty-three cases of secondary cancer of the spleen are described. The splenic neoplasms may or may not be recognized grossly. Microscopically, they represent the parent tissue. The reasons advanced for the paucity of secondary neoplastic involvement of the spleen are based on the mechanistic and antineoplastic peculiarities of the spleen. The latter has brought forth much work, discussion and disagreement. For the present it is best to consider the question of the antipathy of the spleen toward neoplasms as unanswered.

Definite splenomegaly occurs in some of the cases.

237 South Forty-Eighth Street.

Jefferson Medical College Hospital.

11. Kraft, cited by Dial.[7]

Case Reports

RETROPERITONEAL SYMPATHICOBLASTOMA WITHOUT INVOLVEMENT OF THE ADRENAL GLANDS

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Neoplasms of the sympathetic system form an important group of the tumors encountered in infancy and childhood. Among 100 consecutive malignant tumors in young children Blacklock [1] found 12 which arose from sympathetic nerve structures.

Owing to the vast distribution of sympathetic nerve tissue, these neoplasms may develop almost anywhere within the body, but most often they are found in the retroperitoneal region, in or adjacent to the adrenal glands. For a long time they were diagnosed as sarcoma, until Marchand [2] demonstrated the similarity in histologic structure between the developing sympathetic nervous system and adrenal tumors. Still in 1907, when Hutchison [3] described tumors of the adrenal gland with metastases to the skull, he classified them as sarcoma.

It remained for Wright [4] to establish definitely their neurogenic origin. He showed that most, if not all, retroperitoneal round cell tumors in young children have the same embryologic origin as the medulla of the adrenal gland and the adjacent sympathetic ganglions.

The great majority of these tumors that have been reported in the literature originated from the medullary portion of the adrenal gland. Relatively few have been described that did not involve the adrenal gland. To this rare group of tumors belong the two which we had the opportunity to observe.

REPORT OF CASES

CASE 1.—A white girl aged 7 weeks entered St. Francis Hospital, Nov. 6, 1939. At her birth a bluish-colored growth had been noted on the left side of the sternum. It was diagnosed as angioma. Following irradiation, this lesion disappeared, but recurred two weeks later. When the infant was 5 weeks old, projectile vomiting developed together with generalized convulsions. There was enlargement of the cranium with bulging of the fontanel and paralysis of the left abducens nerve. Ventriculographic study showed a marked degree of general internal hydrocephalus. The cerebrospinal fluid was under increased pressure and was bloody, and the supernatant fluid was yellow. The Wassermann test of the fluid was negative, and cultures were sterile. The globulin was markedly increased, and the cell count was 2,500, with 84 per cent small round cells, which had the appearance of lymphocytes. A diagnosis of tumor in the posterior fossa was made. Because of the poor physical condition of the infant, operation seemed not advisable. The infant died at the age of 8 weeks.

From the Department of Neurology and Pathology of the St. Francis Hospital.

1. Blacklock, J. W. S.: J. Path. & Bact. **39**:27, 1934.
2. Marchand, F.: Virchows Arch. f. path. Anat. **81**:477, 1880.
3. Hutchison, R.: Quart. J. Med. **1**:33, 1907.
4. Wright, J. H.: J. Exper. Med. **12**:556, 1910.

Necropsy.—On removal of the calvarium, 50 cc. of clear yellow fluid escaped from the subdural space. The brain was enlarged, and the convolutions were flattened. Without ventricular fluid, the brain weighed 490 Gm. At the base, the leptomeninx which covered the cerebellum and medulla was markedly thickened, and there were firm round bluish nodules. The latter varied in size between 2 and 4 mm. and extended from the pia-arachnoid into the cortical brain tissue. There were also a few single nodules on the surface of the pons, of both frontal lobes and of the left parietal lobe.

The ventricles were markedly dilated and filled with light yellow fluid. In the choroid plexus of the right lateral ventricle, a bean-shaped purplish nodule

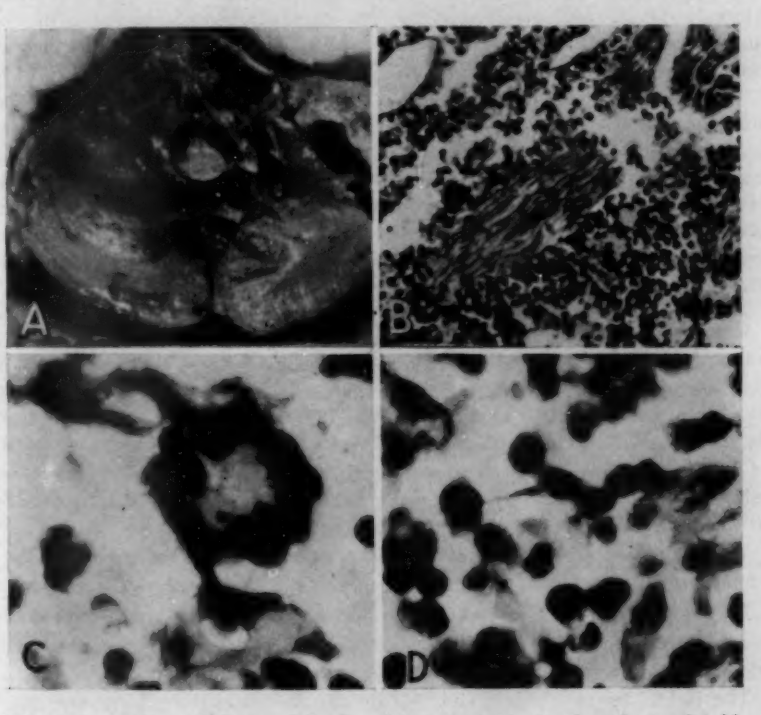

Fig. 1 (case 1).—*A*, base of brain with metastases in the pia-arachnoid and the cortex of the cerebellum and the pons. *B*, metastasis in the cortex of the brain. Nerve fibrils are surrounded by vascular tumor. *C*, pseudorosette of sympathogonia (primary tumor). *D*, sympathoblasts with short fibrils. Bielschowsky's silver stain.

was found. It had a diameter of about 1 cm. The brain tissue surrounding the ventricles was atrophic. There were no tumors in the white matter of the hemispheres or in the basal ganglions. The bones of the skull were free from tumor masses.

In the skin over the sternum, three small bluish black nodules were found. They had not invaded the bone. The heart, the lungs and the mediastinum did not reveal any pathologic changes.

There was no fluid in the abdominal cavity. The liver was enlarged and weighed 258 Gm. It was studded with many small round purplish nodules, which measured between 1 and 3 mm. Retroperitoneally, in the region of the celiac plexus lay a cylindric mass, 4 cm. in length and 1.5 in width. It was attached to the first lumbar vertebra but did not invade the bone. The tumor was situated above the pancreas and had invaded this organ for 1 cm. Sympathetic nerves could be traced into and were lost in the tumor. Both adrenal glands were of normal size, and cross section did not reveal any pathologic changes. The kidneys and the intraperitoneal organs, except the liver, were normal.

Histologic examination of the retroperitoneal tumor and of the metastatic nodules in the skin, the liver, the pia-archnoid and the brain revealed identical pictures. The lesions were made up of groups and strands of small round cells and a great number of thin-walled blood vessels. There were extensive hemorrhages.

The main type of tumor cell had a large spherical hyperchromatic nucleus, while the cytoplasm was indefinite. The average size of the main tumor cells was 7 to 8 microns. In some of the sections, the tumor cells seemed to clump into groups, and occasionally pseudorosettes were present. While most tumor cells were of small size, in many sections somewhat larger cells were encountered, with a lighter staining nucleus and a more distinct cytoplasm. Very few fibrils were found between the tumor cells, some of which stained with Bielschowsky's silver method.

Case 2.—A white girl aged 4 years was admitted April 9, 1940, because the mother had noticed in the child an enlargement of the abdomen. There was constipation, which did not respond to laxatives, and considerable loss of weight. There was moderate right exophthalmos. The abdomen was markedly distended, and there was edema of both lower extremities. A large nodule was found in the left inguinal region, with a scar from previous biopsy. A tentative diagnosis of lymphosarcoma had been made by the family physician. In the lower left quadrant of the abdomen a hard mass could be palpated.

The patient became progressively weaker, the tumor in the groin and in the abdomen did not respond to high voltage therapy, and death occurred June 27.

Necropsy.—The child was extremely emaciated. The right eye showed proptosis. The abdomen was much distended, and the superficial veins of the anterior abdominal wall were greatly dilated.

The right lung weighed 86 Gm. and the left 98 Gm. Many large firm whitish tumors were protruding over the surface of both lungs. The size varied from 2 mm. to 4 cm.

The abdominal viscera were displaced upward by a large firm mass filling out the pelvis. This tumor was located retroperitoneally and was adherent to the sacrum. It was continuous with a smaller nodular mass extending under Poupart's ligament into Scarpa's triangle of the left thigh. Here the tumor encased the femoral vessels and invaded the muscles. The entire dumbbell-shaped mass weighed 1,250 Gm. The cut surface was white, homogeneous and solid with exception of few cystic spaces. No tumor nodules were found in the liver, spleen, kidneys or adrenal glands.

Histologic examination of the pelvic tumor revealed thick strands of round cells separated by fibrous stroma. In sections of the lower portion of the mass extensive necrosis was found.

The main tumor cell measured 8 to 9 microns with a vesicular nucleus of 7 to 8 microns. No distinct nucleoli were distinguishable. The protoplasm of the tumor cells was almost unstained. Mitoses were infrequent. Very few pseudorosettes were found. The scanty protoplasm of the large cells sometimes extended

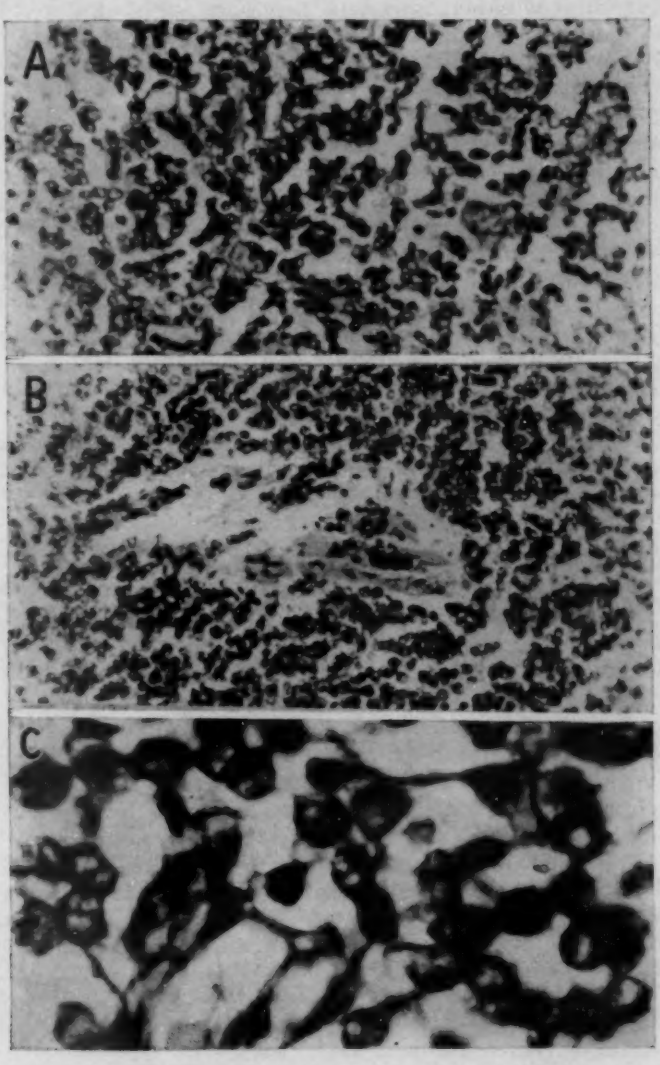

Fig. 2 (case 2).—*A*, primary tumor showing pseudorosettes scanty and indistinct. *B*, pulmonary metastasis. The central area contains fibrils. *C*, fibrils originating from the cytoplasm of sympathoblasts. Bielschowsky's silver stain.

into a short process. Some of the tumor cells were polygonal, of larger size and with more distinctly stained protoplasm. The nodules in the lungs had the same characteristics as the primary tumor. Few fibrils could be demonstrated by silver stain.

INCIDENCE OF SYMPATHICOBLASTOMA

The tumors that are essentially those of infancy and early childhood are neuroblastoma of the retina, Wilms's tumor of the kidney and sympathicoblastoma. There is no evidence that heredity plays any role in the occurrence of either malignant nephroma or sympathicoblastoma, whereas there is no other malignant tumor in which heredity plays so evident a role as in retinoblastoma. For this discrepancy no explanation is apparent.

The growth in our first case belongs to the little known group of congenital extra-adrenal sympathicoblastoma. Wells [5] found in the literature only 3 cases of congenital sympathicoblastoma, in each of which the neoplasm arose in the abdominal portion of the sympathetic nervous system. Regardless of age, retroperitoneal sympathicoblastoma without involvement of the adrenal medulla is of rare occurrence. In the series of Stern and Newns [6] only 4 of 25 cases were without adrenal origin of the tumor. Harbitz [7] observed in a 3 year old child a neoplasm which was located anteriorly to the sacrum and originated in the sacral sympathetic plexus. Schultz [8] reported a tumor which arose from the left abdominal sympathetic trunk. Wahl and Craig [9] described in a 28 year old Negro three independent primary tumors arising from the lower end of the sympathetic system, each different in structure and representing three stages in the differentiation of the malignant sympathicoblastoma to the benign ganglioneuroma.

CLINICAL DIAGNOSIS

The clinical symptoms of sympathicoblastoma are as a rule of great variety and are often confusing, owing to the presence of metastases almost anywhere in the body. Because of the multiplicity of symptoms, the pediatrician, the internist and the abdominal surgeon may encounter these tumors as well as the urologist and the neurologist. In most cases the metastases produce the first manifestation of the disease. The organs most commonly affected by way of the lymphatic system or of the blood stream are the liver, the bones of the skull, especially in the orbital region, the kidneys and the lungs.

The brain is rarely the seat of metastases. In the literature we found reports of only 2 cases with cerebral involvement. Gareiso [10] observed, in an 11 year old child, metastasis directly to the hypophysis, and Dimitri and Alem [11] reported an adrenal tumor with a metastatic nodule in the white matter of the brain in a man 47 years of age. In our first case the whole clinical picture was governed by the secondary tumor nodules

5. Wells, H. G.: Arch. Path. **30**:535, 1940.

6. Stern, R. O., and Newns, G. H.: Arch. Dis. Childhood **12**:267, 1937.

7. Harbitz, F.: Arch. Int. Med. **16**:312, 1915.

8. Schultz, O. T., in Abt, I. A.: Pediatrics, Philadelphia, W. B. Saunders Company, 1926, vol. 8, p. 744.

9. Wahl, H. R., and Craig, P. E.: Am. J. Path. **14**:797, 1938.

10. Gareiso, A.; Vergnolle, M. J., and Petre, A. J.: Rev. méd. latino-am. **21**:31, 1935.

11. Dimitri, V., and Alem, C.: Rev. neurol. de Buenos Aires **3**:33, 1938.

in the brain and in the pia-arachnoid. The primary tumor in the celiac plexus had not produced any symptoms, and the clinical diagnosis was tumor of the brain.

Our first case is similar to the one reported by Blacklock, that of a boy aged 1 year and 6 months, with the diagnosis of acute mastoiditis. Operation disclosed a soft vascular tumor in the region of the mastoid, which microscopically had the characters of sympathicoblastoma. At autopsy both adrenal glands were normal; the primary tumor was found in the region of the celiac plexus. While there were extensive metastases in the bones of the skull, at no place had the tumor extended into the brain.

Tumors arising from the sympathetic ganglions in the lumbar and thoracic areas may invade the spinal canal and produce symptoms of root and spinal cord involvement. In 4 cases of paravertebral sympathicoblastoma reported by Chandler and Norcross [12] the following clinical diagnoses were made: paraplegia due to encephalomyelitis, tumor of the spinal cord, tuberculoma of the mediastinum and Pott's disease.

In our second case the first symptom was produced by direct extension of the retroperitoneal sympathicoblastoma into the left inguinal region. The physician regarded the swelling in the groin as lymphosarcoma and advised biopsy. Microscopic study revealed the structure of sympathicoblastoma. Of the tumors recorded in the literature, the only one which had a similar localization is that reported by Ewing.[13] In a child of 2 years, a tumor appeared first on the inner side of the left thigh below Poupart's ligament and grew rapidly to enormous dimensions. It invaded and filled the pelvis, and by way of the retroperitoneal lymphatics it invaded both lungs. The liver was entirely free from metastases.

HISTOLOGIC CHARACTER OF TUMORS OF SYMPATHETIC NERVE TISSUE

According to Kohn,[14] neuroblasts in early embryonal life emigrate from the ganglionic crest of the medullary tube along the anterior spinal segmental nerves and form the various sympathetic plexuses and the medulla of the adrenal gland. These neuroblasts divide and form sympathogonia, which are the formative cells of the sympathetic nervous system. They are thus analogous to the medulloblasts of the central nervous system. The sympathogonia are small, darkly staining cells with little cytoplasm. They resemble lymphocytes and are usually arranged in clusters and imperfect rosettes. According to Bielschowsky,[15] they form delicate fibrils, which are not demonstrable by the silver methods. The sympathogonia destined to form the large sympathetic ganglions ultimately develop into ganglion cells through an intermediate

12. Chandler, F. A., and Norcross, J. R.: J. A. M. A. **114**:112, 1940.

13. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 836.

14. Kohn, A.: Arch. f. mikr. Anat. **70**:266, 1907.

15. Bielschowsky, M., in Penfield, W.: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932 vol. 3, p. 1085.

cell stage in which they are termed sympathoblasts. The latter are larger than the sympathogonia and have more vesicular nuclei and more abundant protoplasm; they produce the early sympathetic nerve fibrils which can be stained by the silver method.

The tumors of the sympathetic system have been classified by Landau,[16] Robertson[17] and Scott[18] according to the degree of differentiation of their cells. For example, those formed of sympathogonia have been classified as sympathogonioma and those of ganglion cells as ganglioneuroma. No tumor composed only of sympathoblasts has as yet been reported. Any attempt at rigid classification is rendered difficult by the fact that different stages of development may be present in the same tumor; i. e., sympathogonia, sympathoblasts and ganglion cells may all be found together (Wahl[19]; Dunn[20]; Herxheimer[21]; Potter and Parrish.[22]).

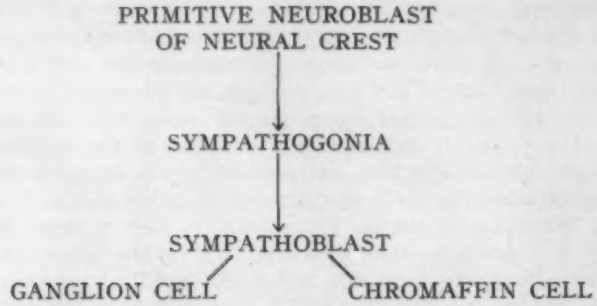

Fig. 3.—Development of the cells of the sympathetic nervous system.

Bailey and Cushing[23] suggested for undifferentiated tumors of the sympathetic system the term "sympathicoblastoma"; and Blacklock[1] divided these neoplasms into the following groups:

I. Sympathicoblastoma (all malignant)
  A. Undifferentiated (composed of sympathogonia only)
  B. Differentiated
    1. Composed of sympathogonia and sympathoblasts
    2. Composed of ganglion cells in addition to more primitive cells (gangliosympathicoblastoma)

II. Ganglioneuroma (generally benign) composed only of mature ganglion cells.

The histologic structure of our two tumors places them in Blacklock's first subgroup of differentiated sympathicoblastoma; most of the cells

16. Landau, M.: Frankfurt. Ztschr. f. Path. **11**:26, 1912.
17. Robertson, H. E.: Virchows Arch. f. path. Anat. **220**:147, 1915.
18. Scott, E.; Oliver, M. G., and Oliver, M. H.: Am. J. Cancer **17**:396, 1933.
19. Wahl, H. R.: J. M. Research **30**:205, 1914.
20. Dunn, J. S.: Glasgow M. J. **84**:98, 1915.
21. Herxheimer, G.: Beitr. z. path. Anat. u. z. allg. Path. **57**:112, 1914.
22. Potter, E. L., and Parrish, J. M.: Am. J. Path. **15**:653, 1939.
23. Bailey, P., and Cushing, H.: A Classification of Tumors of the Glioma Group, on a Histogenetic Basis, Philadelphia, J. B. Lippincott Company, 1926.

were sympathogonia and only occasional fibrils could be demonstrated with silver methods. However, in both tumors some sympathoblasts were present. The principal tumor cell in the first was slightly smaller in size than that in the second. The tendency to formation of pseudo-rosettes was more evident in the first tumor.

The fact that in both tumors only few fibrils stained with Bielschowsky's silver method corresponds to the experience of Blacklock, while Pick [24] and Bielschowsky [15] were able to show, by means of silver stain, that the great bulk of the fibrillar material in sympathicoblastoma is composed of naked axis-cylinders. Landau [16] secured excellent results with the iron-hematoxylin and Van Gieson's stain. In our first tumor very delicate fibrils stained with hematoxylin-eosin were occasionally traced from the tail-like cytoplasmic process of the tumor cells.

SUMMARY

Two cases of retroperitoneal sympathicoblastoma without involvement of the adrenal glands have been reported.

The multiplicity of symptoms was due to the metastases, and the primary tumor was overlooked before death.

One case belongs to the rare group of congenital sympathicoblastoma. In this case the clinical picture was governed by metastases to the brain and the pia-arachnoid. Metastasis of sympathicoblastoma directly to the brain of an infant has never been reported previously.

24. Pick, L.: Berl. klin. Wchnschr. **49**:16 and 76, 1912.

MIXED TUMOR OF THE PAROTID WITH METASTASES

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Passed Assistant Surgeon, United States Public Health Service

Mixed tumors of the salivary glands frequently recur after removal, and they may become locally invasive and involve regional lymph nodes. Distant metastases from these tumors, however, are considered to be quite rare.

In 1924 Porter and Churchill [1] collected from the literature reports on 7 malignant tumors which they considered to be of mixed tumor origin and from which metastasis had taken place through the blood stream. McFarland [2] reviewed the literature in 1926 for metastasizing mixed tumors of the salivary glands and listed 9 such tumors; 4 of these were included in the 7 reported by Porter and Churchill. In the 12 cases of both reports, the original tumors were variously diagnosed, and often their true nature was not clear.

Since 1926, several mixed tumors with distant metastases have been reported. Charbonnel and Chabé,[3] Fitzwilliams,[4] McFarland,[5] Montanus,[6] Kornblith [7] and Livingston [8] each described 1 case, while Olson [9] reported 3 cases. The presence of metastatic lesions was determined by roentgen examination alone in 4 of these cases, and final proof as to the exact nature of the lesions was lacking.

In a majority of the reported cases of mixed tumors with distant metastases, there were one or more recurrences of the primary tumor before the metastatic lesions were discovered. In some of the cases the metastases were described histologically as mixed tumors, but in a majority they were diagnosed as metastatic carcinoma or sarcoma. In this regard, many authors believe that metastasizing mixed tumors undergo "carcinomatous" or "sarcomatous degeneration" before metastases develop, and that the latter are either carcinoma or sarcoma, depending on the type of "degeneration" occurring in the original tumor.

From the Division of Pathology, National Institute of Health, United States Public Health Service.

1. Porter, C. A., and Churchill, E. D.: Surg., Gynec. & Obst. **38**:336, 1924.
2. McFarland, J.: Am. J. M. Sc. **172**:804, 1926.
3. Charbonnel and Chabé: Bordeaux chir. **4**:58, 1933.
4. Fitzwilliams, D. C. L.: Lancet **2**:769, 1935.
5. McFarland, J.: Surg., Gynec. & Obst. **63**:457, 1936.
6. Montanus, W. P.: Surgery **4**:423, 1938.
7. Kornblith, B. A.: J. Mt. Sinai Hosp. **6**:38, 1939
8. Livingston, S. K.: Am. J. Roentgenol. **44**:887, 1940.
9. Olson, G. W.: Laryngoscope **47**:252, 1937.

The case to be presented is that of a mixed tumor of the parotid gland with multiple distant metastases. The case differs from a majority of those previously reported in that metastases were discovered shortly after removal of the primary tumor and histologic examination of the original and the metastatic lesions revealed mixed tumor of the salivary gland type.

REPORT OF A CASE

A white man, 35 years of age, entered the United States Penitentiary Annex, Fort Leavenworth, Kan., in 1938. Shortly after admission, he applied for treatment of a tumor under the right ear. He had first noticed a firm sensitive swelling under the ear in 1931. This had gradually enlarged, and in 1936 he began to use narcotics because of constant pain in the ear. However, he sought no medical attention until he entered the penitentiary. At this time there was partial paralysis of the right side of the face, and the patient complained of frequency of urination and pains in the lumbar region.

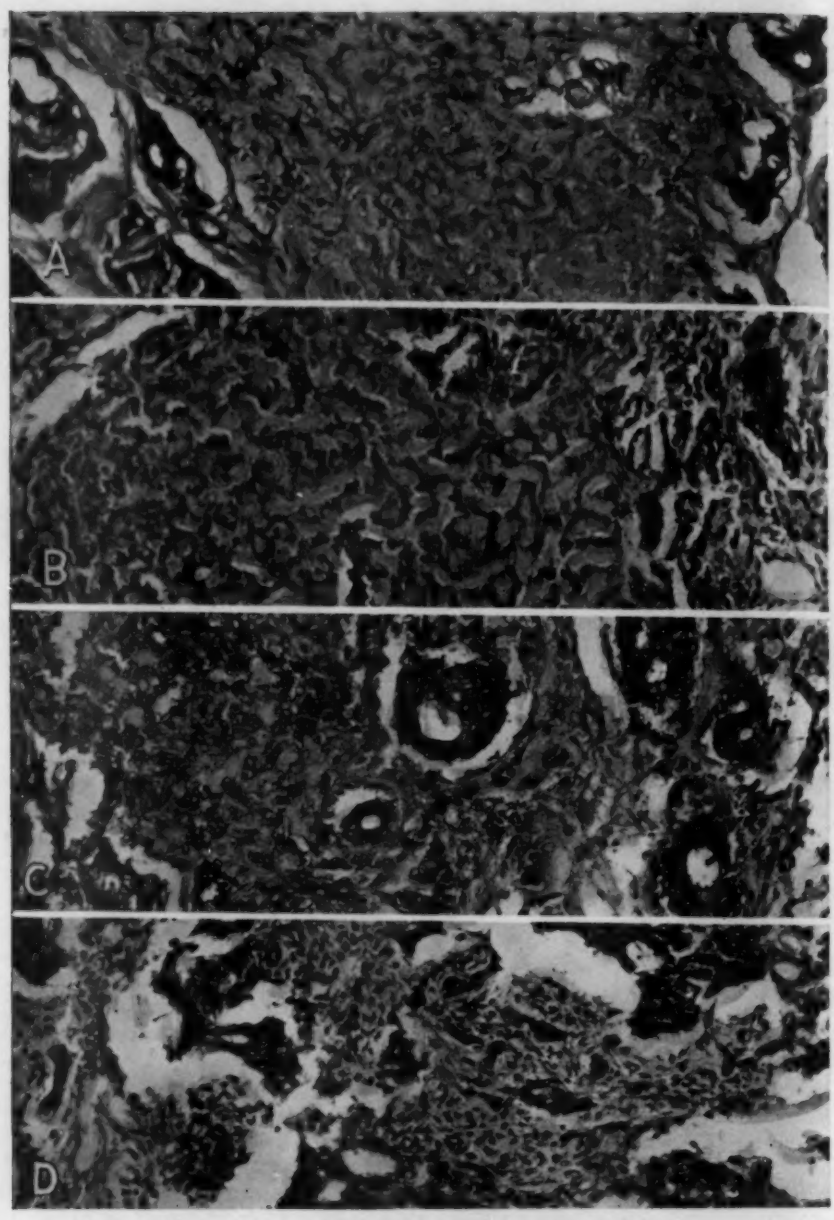
The tumor was removed on Nov. 2, 1938. Operative data are not available. The report of the microscopic examination, done by Passed Assistant Surgeon J. G. Pasternack, of the United States Marine Hospital, New Orleans, is as follows: "The specimen presents a matrix of hyalin and mucocartilage. Through this matrix there are cell strands and aggregates of glands formed of juvenile cubical epithelium. In areas, hollowed and branching formations of neoplastic epithelium with hyperchromatic nuclei are present. This specimen is more parenchymatous than tumors of this type usually are. These tumors are locally malignant and can readily recur if removal is incomplete. Distant metastases rarely occur. Diagnosis: Chondrocarcinoma of the right parotid gland."

Immediately after the operation, the partial facial paralysis became complete. The pain in the lumbar region gradually increased in severity, and about one month after operation the patient's legs became weak. Soon thereafter his legs became completely paralyzed, and roentgen pictures of the lumbar region revealed a defect in the third lumbar vertebra. The patient was transferred to the Medical Center for Federal Prisoners at Springfield, Mo., in January 1939.

Examination at this time by Dr. C. S. Sample revealed complete paralysis of the right side of the face. An operative scar in the region of the right parotid gland was well healed, and there was no local evidence of recurrence of the growth. There were complete flaccid paralysis of both legs with lack of sensation except for the outer and upper surfaces of the thighs; the patient was unable to void. Multiple round, firm subcutaneous nodules, measuring from 1 to 2 cm. in diameter, were present over the abdomen, the chest and the upper surfaces of the thighs. There was kyphosis at the level of the third lumbar vertebra and a dense mass could be felt to the right of the vertebral column at this level. Laboratory examination revealed moderate secondary anemia and the urine contained albumin (2+) together with many white blood cells and a few erythrocytes.

The patient appeared to be hopelessly ill, his condition grew progressively worse, and he died on March 12, 1939. An autopsy was done by Dr. Murray Stone, consulting pathologist, twenty-one hours after death.

At autopsy there were many subcutaneous tumor nodules measuring from 1 to 3 cm., and several similar nodules were found in the omentum; the nodules were usually firm and pinkish white on section. Large retroperitoneal tumor masses were present on each side of the vertebral column in the vicinity of the second, third and fourth lumbar vertebrae, which were largely destroyed. Irregular



A, primary tumor showing hyalinized stroma; × 155.

B, metastatic pulmonary lesion showing structure similar to that shown in *A*; × 155.

C, primary tumor showing epithelial cell nests; × 155.

D, metastatic pulmonary lesion showing epithelial cell nests and cords in cellular stroma; × 155.

masses and small nodules of tumor were scattered over the surfaces and in the parenchyma of both lungs; the largest mass was about 5 cm. in diameter. In the liver, two tumor nodules were seen on the surface, and a third was observed in the parenchyma of the right lobe. Both kidneys were separable from the retroperitoneal tumor masses, although the left kidney was displaced by the growth and resting in a horizontal position; no tumor was noted in the kidneys. The adrenals were not demonstrable. The right testicle was globular and hard, and the parenchyma was largely replaced by an encapsulated structure which was filled with a yellow cheesy substance. The left testicle was normal. The heart, spleen, gallbladder, intestines and pancreas were normal.

Tissue was removed from one of the retroperitoneal tumor masses, the lungs, the liver and the testicle and sent to the National Institute of Health, Washington, D. C., for histologic examination.

Microscopic Examination of the Tumor.—The tumor nodules in the lung and the liver were sharply circumscribed and similar in structure to the retroperitoneal tumor. The tumor was composed of irregular nests and cords of epithelial cells and few small acini set in a loose fibrillar to moderately dense and collagenous stroma; epithelial elements predominated in some areas and stroma in others. For the most part the stroma was moderately cellular, and the stromal cells were chiefly spindle shaped and of the fibroblast type; intercellular mucoid material was present in a few areas, and hyalinization was noted in others. The epithelial cells in the nests and cords were chiefly polyhedral, while those in the acini were cuboidal; nuclei were round to oval and trachychromatic, and small nucleoli were often seen. Mitoses were present in small to moderate numbers. Some of the epithelial cell nests and cords were sharply circumscribed but others had the peripheral cells loosely arranged and merged into the stroma.

The testicle showed a subacute tuberculous process involving the epididymis and vas deferens.

The final diagnosis in the case, based on microscopic examination of the autopsy material and of slides from the original tumor, was mixed tumor of the parotid gland, with metastases in the lung, the liver and the retroperitoneal tissue. Tissues from the subcutaneous, omental and vertebral lesions were not available for histologic study.

COMMENT

The clinical course of the original tumor was consistent with that of mixed tumors in regard to duration and rate of growth; there was gradual increase in size over a period of seven years. Involvement of the facial nerve with production of pain five years after onset and partial paralysis two years later was suggestive of local invasiveness.

Histologically, the original tumor was of the mixed tumor type and, although it was quite cellular, it was not frank carcinoma.

Certain subjective symptoms were present at the time of operation, and less than two months after operation the metastatic lesions responsible for these symptoms were detected. The metastases must have been present at the time of operation, and yet there were no definite clinical or histologic criteria to indicate such a possibility. Moreover, the metastases developed from a mixed tumor which had not been subjected to surgical trauma through prior attempts at removal.

The histologic structure of the metastatic lesions was of the mixed tumor type and was similar to the original tumor in many respects; there was no histologic evidence that "carcinomatous" or "sarcomatous degeneration" had taken place before or during development of metastases.

SUMMARY

A case of mixed tumor of the parotid with multiple distant metastases is reported. After removal of the original tumor, certain developments indicated that metastasis had occurred at the time of operation, although there were no definite clinical or histologic criteria to indicate such an event. The metastatic lesions presented the histologic structure of mixed tumor of the salivary gland type.

Laboratory Methods and Technical Notes

COMBINED FROZEN AND PARAFFIN METHOD FOR RAPID SECTIONS

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Rapid sections prepared by the ordinary frozen section method during the course of surgical operations frequently fail to give sufficient histologic detail for positive diagnostic conclusions. To meet the demand for a more satisfactory section that can be prepared in a relatively short time, the method described in detail herein has been devised.

This method has been in use in this department for several months, and the results have been gratifying. In fact, the sections have been so satisfactory that the method is now being used on many occasions on which there is no need of a prompt report to the surgeon but merely a desire on the part of the staff to get a good histologic section without unnecessary delay.

The method, which is a combination of technics for the preparation of frozen and paraffin sections, enables one to prepare in approximately twenty minutes a permanent cleared section that is comparable in a general way with the routine hematoxylin-eosin-stained paraffin preparation. If other staining methods are desired, the procedure given here may be readily modified.

TECHNIC

1. The tissue, if not already fixed, is placed in hot dilute formaldehyde solution (10 cc. of solution of formaldehyde U. S. P. in 90 cc. of water) for three minutes. The solution should be steaming (about 65 C.) but not boiling, and the pieces of tissue may be large, but not over 3 mm. in thickness.

2. Frozen sections, as thin as possible (10 to 15 microns), are prepared by the usual method. Overfreezing should be avoided as it causes an unnecessary delay.

3. Sections are transferred from the knife to a dish of warm (40 C.) 0.2 per cent gelatin solution, from which a section is then floated carefully onto a clean slide, and any wrinkles that may form are teased out.

4. The excess fluid around the section is wiped off, and the slide dried cautiously over the pilot flame of a bunsen burner. Overheating is to be avoided.

5. When the section is thoroughly dried, it is cooled and placed in formaldehyde alcohol (10 cc. of solution of formaldehyde U. S. P. in 90 cc. of 95 per cent ethyl alcohol) for one-half minute.

6. It is then dehydrated in 95 per cent ethyl alcohol followed by absolute alcohol, one-half minute in each.

7. After being cleared in xylene for one-half minute, it is transferred to a beaker of melted paraffin in an oven at 60 C. for three minutes.

From the Department of Pathology, Royal Victoria Hospital.

8. The slide is then removed from the beaker of paraffin and returned to the oven for five minutes. This assures attachment of the section to the slide during the staining process and apparently produces a mordant effect on the tissues.

Various staining methods may now be employed. The technic for the hematoxylin-eosin method is as follows:

1. Remove paraffin with xylene	30 seconds
2. Place in 95 per cent alcohol	15 seconds
3. Place in 70 per cent alcohol	15 seconds
4. Wash quickly in tap water	10 seconds
5. Stain in fresh Harris hematoxylin	1 minute
6. Wash in water, decolorize in 0.5 per cent aqueous solution of hydrochloric acid, wash and intensify in a weak solution of ammonia water and again wash	30 seconds
7. Counterstain with eosin and rinse in water	1 minute
8. Dehydrate in 70 per cent, 95 per cent and absolute alcohol	10 seconds each
9. Clear in xylene	
10. Mount in Canada balsam	

Under the microscope, sections prepared by this method, in contrast with the usual frozen sections, are clear, with brightly stained and excellent histologic detail.

A NEW MODIFICATION OF PERLS'S REACTION FOR HEMOSIDERIN IN TISSUES

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The original method of demonstrating iron in microscopic preparations is the prussian blue method described by Perls.[1] Many modifications of this method have been proposed [2] because no one of them is perfectly satisfactory.

Hitherto we have used the Abbott modification (Mallory and Wright [3]), which consists in treating sections for forty-five seconds with a mixture of 1 part of 2 per cent aqueous potassium ferrocyanide solution (freshly prepared; i. e., not over a week old) and 3 parts of 1 per cent aqueous hydrochloric acid at 60 to 80 C. Sections are then washed and counterstained in 0.1 to 0.5 per cent basic fuchsin in 50 per cent alcohol for five to twenty minutes.

Two drawbacks were noted. There was a tendency toward slight diffusion of the prussian blue, particularly if the mixture of warm hydrochloric acid and potassium ferrocyanide was allowed to remain on the slide for more than forty-five seconds; and there was a tendency toward overstaining of the cytoplasm by the basic fuchsin when this was used in the recommended dilution. While these tendencies could be controlled to a great extent in the light of experience and careful attention to detail, they were definite drawbacks; accordingly an attempt was made to find a more foolproof technic.

In the experiments described here, sections of a markedly hemosiderotic human lung were used. The tissue was fixed routinely in solution of formaldehyde U. S. P. diluted 1:10. Paraffin sections were brought to water as usual and then treated with the various solutions for varying periods. After counterstaining, they were washed in water, dehydrated, cleared and mounted by successive use of acetone, acetone and xylene mixture, xylene and clarite.

To overcome the tendency toward diffusion of the prussian blue, acetic acid was tried in place of hydrochloric acid, since Lillie [4] had shown that hemosiderotic tissue treated at 37 C. with 20 per cent acetic acid still retains considerable hemosiderin even after forty-eight hours. Dilutions of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 per cent acetic acid were prepared, and 1 volume of each dilution was mixed with an equal volume of a freshly prepared 2 per cent solution of potassium ferrocyanide. Each mixture was heated in a test tube until beads of gas formed

1. Perls, M.: Virchows Arch. f. path. Anat. **39**:42, 1867.

2. Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938. Gömöri, G.: Am. J. Path. **12**:655, 1936.

3. Mallory, F. B., and Wright, J. H.: Pathological Technique, ed. 8, Philadelphia, W. B. Saunders Company, 1924.

4. Lillie, R. D.: Am. J. Path. **12**:655, 1936.

on the inner surface of the glass (about 60-80 C.), poured on the slides, and after forty-five seconds the slides were washed in water and counterstained with dilute basic fuchsin.

From 4 to 6 per cent acetic acid gave the best results. Small granules were blue and larger granules a dark brownish green. This would indicate that only the surface of the coarser granules is transformed into prussian blue. Less than 4 per cent acetic acid gave predominance of the brown color even in many of the smaller granules, and more than 6 per cent produced a slight diffusion of the stain, which formed blue haloes around the granules.

Next the interval of exposure was increased from forty-five seconds to two, five and fifteen minutes. At the end of fifteen minutes, even the 0.5 per cent acetic acid mixture produced a blue reaction. A 4 per cent acetic acid mixture produced only slight diffusion of the prussian blue at the end of fifteen minutes and no significant amount of diffusion at the end of five minutes.

To overcome the tendency to overstaining, basic fuchsin was prepared in dilutions of 1:500, 1:1,000, 1:2,000, 1:3,000, 1:4,000, 1:5,000, 1:6,000, 1:8,000 and 1:10,000 by using as solvents in turn 0.5, 1, 2 and 3 per cent aqueous solutions of acetic acid. Our best results were obtained with solutions of 1:6,000 to 1:10,000 basic fuchsin in a 2 to 3 per cent aqueous solution of acetic acid. This gave excellent nuclear detail in deep red and, sharply contrasting with the prussian blue, an excellent background of pink, which was optimum after five minutes, but which was neither too light after two minutes nor too deep after thirty minutes of staining.

An attempt was then made to combine the prussian blue reaction and the counterstaining in one procedure. One volume of a freshly prepared 2 per cent aqueous solution of potassium ferrocyanide was mixed with an equal volume of dilute basic fuchsin in aqueous acetic acid solution, heated in a test tube as previously described, poured over the tissue sections and allowed to remain for varying intervals. The concentrations of the basic fuchsin so tested were 1:1,000, 1:2,000, 1:3,000, 1:4,000 and 1:5,000; the concentrations of the acetic acid solutions were 4, 5, 6, 8, 10 and 12 per cent; the intervals of exposures were forty-five seconds, two, three, four, five and fifteen minutes. Our best results were obtained by treating the sections for periods of from forty-five seconds to five minutes with a heated mixture of 1 volume of the solution of potassium ferrocyanide and an equal volume of a 1:3,000 solution of basic fuchsin in 6 per cent aqueous acetic acid. However, the results were not uniform, and there was a tendency toward predominance of the greenish brown color even in the smaller granules.

SUMMARY

A new modification of Perls's reaction for hemosiderin in microscopic preparations is proposed.

It is suggested that 1 volume of a 2 per cent aqueous solution of potassium ferrocyanide be mixed with an equal volume of a 4 to 6 per cent aqueous acetic acid, heated in a test tube until beads of gas appear on the inner surface of the glass (about 60-80 C.), poured on the section and allowed to remain thereon for from forty-five seconds to five minutes.

After being washed in water, the section is counterstained five minutes with a 1:6,000 to 1:10,000 solution of basic fuchsin in 2 per cent aqueous acetic acid.

Forensic Medicine

ANTEMORTEM AND POSTMORTEM DIFFUSION OF ALCOHOL THROUGH THE MUCOSA OF THE BLADDER

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Alcohol passes from the blood to the urine by diffusion, and the difference in its concentration in simultaneously collected specimens of arterial blood and ureteral urine probably corresponds to the difference in the water content of the two fluids.[1] Jetter [2] has observed that the ratio expressing the amount of alcohol per unit volume of ureteral urine divided by the amount of alcohol per unit volume of arterial blood varies inversely with the specific gravity of the urine and that with a urine at a specific gravity of 1.010 the ration was approximately 1.37. The higher the specific gravity of the urine the less was the difference between the concentration of alcohol in the urine and that in the blood, and for each increase of 0.010 in specific gravity between 1.010 and 1.040 the value expressing the ratio of the concentration of alcohol in ureteral urine to the concentration of alcohol in arterial blood was diminished by approximately 0.07. Thus at a specific gravity of 1.020 the ratio was approximately 1.30; at 1.030 it was approximately 1.23, and at 1.040 it was approximately 1.16.

If the concentration of alcohol in the ureteral urine bears a more or less fixed relation to that in the blood and if the urine of the bladder represents an unaltered pool of ureteral urine, the latter should be a potential source of information of both clinical and medicolegal significance. By dividing the number of grams of alcohol per hundred cubic centimeters of a sample of urine from the bladder known to have been secreted over a definite interval of time by the appropriate $\frac{\text{urine alcohol}}{\text{blood alcohol}}$ ratio one should obtain a value which would be representative of the

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1. Haggard, H. W., and Greenberg, L. A.: J. Pharmacol. & Exper. Therap. **52**:150, 1934.

2. Jetter, W. W.: Quart. J. Stud. on Alcohol **2**:512, 1941.

mean concentration of alcohol in the blood for the interval during which the urine had been formed.

Even though the exact duration of the period of collection were not known, it might be possible to infer whether the level in the blood had been previously rising or falling by comparing the concentration of the alcohol in the urine of the bladder with that in a simultaneously obtained specimen of blood. Thus, if the mean concentration of alcohol in the blood as interpolated from the concentration of alcohol in the urine of the bladder were found to be higher than that of the alcohol in the arterial blood, it could be inferred that the blood level had actually been higher during the time that the urine of the bladder was collecting than it was when the specimens of urine and blood were obtained for analysis. If the reverse were found, it could be inferred that the blood level had been previously lower.

The only time that the blood alcohol level undergoes rapid change is during the period of active absorption when it may rise from 0 to 0.4 Gm. per hundred cubic centimeters or even higher within a few hours' time. A much longer period is usually required for the accomplishment of a corresponding descent in the blood alcohol curve. With respect to tests performed for medicolegal purposes, it may be claimed that the information derived by testing samples obtained several hours after an accident or a traffic violation is not reliable. If the urine of the bladder represents an unaltered pool, such an allegation might be supported or impunged by comparing the concentrations of alcohol in simultaneously collected specimens of blood and urine. If it were true that the blood alcohol had been rising during the period that preceded the obtaining of test specimens, it would be reasonable to expect that the concentration of alcohol in the urine of the bladder would be significantly lower than that in a simultaneously obtained sample of blood. If the concentration of alcohol in the urine of the bladder were found to be disproportionately high, it could be inferred that the blood level of the test subject had been higher rather than lower during the period that preceded the obtaining of the specimen.

It is obvious, however, that before any inferences can be drawn from the concentration of alcohol in the urine of the bladder or from a comparison of this concentration with that of alcohol in the blood, it must first be determined whether or not the urine of the bladder is in reality an unaltered pool of ureteral urine. If it is, the urine of the bladder is potentially a rich source of useful information as to whether the blood level was rising or falling prior to the time the test specimen was collected. If it is not, it would be desirable to have more information than is now available as to the extent and the circumstances in which the composition of ureteral urine is altered in the bladder.

That a direct interchange of alcohol between the urine and the blood may occur through the mucosa of the bladder was first observed by Voltz, Baudrexel and Dietrich,[3] in 1912. This fact was confirmed by Nicloux and Nowicka,[4] who showed that the interchange may occur in either direction, depending on the magnitude of the initial difference in concentration of alcohol between the two fluids. They found that when the blood contained considerably more alcohol than the urine of the bladder, alcohol passed through the mucosa of the bladder from the blood to the urine; when the reverse was true, diffusion occurred in the opposite direction.

In 1940 Haggard, Greenberg, Carroll and Miller[5] called attention to the fact that the differences which existed between the initial concentration of alcohol in the urine of the bladder and that in the blood of the animals used in the earlier experiments were far in excess of those likely to be encountered in man. In order to determine whether or not alcohol may be absorbed from the bladder in significant amounts under physiologic conditions, they conducted two series of experiments.

In the first of these, 2 human subjects drank 180 cc. of water containing 40 Gm. of alcohol each day for ten days. On five alternate days urine was voided at thirty minute intervals during the first eight hours after the alcohol had been ingested. On the other five days the urine was held in the bladder for the entire eight hour period. From one subject the urinary output of alcohol when voided at thirty minute intervals was 20.78 cc., compared with 20.10 cc. when the urine remained in the bladder for eight hours. From the other subject the urinary output when voided at thirty minute intervals was 25.92 cc., compared with 25.76 cc. when the urine remained in the bladder throughout the eight hour postingestion period.

In the second series of experiments conducted by Haggard and his collaborators the 2 test subjects took no alcohol by mouth. Urine containing a measured amount of alcohol was introduced by catheter into the recently emptied bladder of each subject and allowed to remain there for two hours. In one the total loss of alcohol due to absorption, diffusion or artefact was 5 per cent, and in the other it was 7 per cent.

On the basis of these two sets of observations on each of 2 human subjects it was concluded that the absorption of alcohol from the urinary bladder in man is of no practical significance at the concentrations in which it is likely to be encountered in ordinary testing.

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3. Voltz, W.; Baudrexel, A., and Dietrich, W.: Arch. f. d. ges. Physiol. **145**: 186, 1912.

4. Nicloux, M., and Nowicka, V.: J. de physiol. et de path. gén. **15**:296, 1913.

5. Haggard, H. W.; Greenberg, L. A.; Carroll, R. P., and Miller, D. P.: J. A. M. A. **115**:1680, 1940.

No exception can be taken to the conclusions of Haggard and his collaborators so far as they apply to the experimental conditions in which they were made. It was felt, however, that the results of these two sets of experiments did not justify the assumption that the urine of the bladder is invariably an unaltered pool of ureteral urine. The following experiments were undertaken for the purpose of investigating more fully the circumstances and the extent to which the alcohol content of the urine of the bladder may be modified by diffusion through the mucosa of the bladder.

EXPERIMENTS

Experiment 1. Passage of Alcohol into the Urine by Way of the Mucosa of the Bladder in Living Dogs.—Alcohol was administered intravenously in the form of a 20 per cent solution in physiologic solution of sodium chloride to 4 dogs in amounts sufficient to bring the concentration of alcohol in the blood above 0.3 per cent. At the beginning of the injection the dogs received enough soluble pentobarbital to prevent struggling, and additional soluble pentobarbital was given when necessary. About one hour after completion of the injection of alcohol, the abdomen was opened, both ureters were ligated and cannulas were inserted into their renal ends for the dual purpose of insuring that no urine would enter the bladder and of preventing the escape of urine into the peritoneal cavity.

The operative procedure described was carried out in all the dogs used in this and succeeding experiments. After the bladder had been thus isolated from the kidneys, its contents were withdrawn by urethral catheter, after which the bladder was washed thoroughly with physiologic solution of sodium chloride and between 50 and 100 cc. of urine made up to an alcohol concentration considerably lower than that of the blood was injected back into the bladder and kept there by means of a clamped retention catheter.

Thereafter samples of blood and urine were taken simultaneously at arbitrary intervals. Each pair of samples was comprised of 5 cc. of blood obtained by cardiac puncture and 2 cc. of urine aspirated from the bladder through an inlying urethral catheter. Before removal of each sample of urine, the urine was flushed back and forth through the catheter so as to make sure that the sample was representative of the content of the bladder. The flasks in which the blood was received contained sodium fluoride and potassium oxalate, and those in which the urine was received contained sodium fluoride. All samples were analyzed for alcohol by Harger's method.[6]

The results of the first set of experiments are shown in table 1. The total amount of fluid removed from the bladder of each animal was approximately the same as that originally introduced. In each instance the initial concentration of alcohol in the blood was considerably higher than that in the urine of the bladder. In all 4 dogs the concentration of alcohol in the blood fell continuously through the period of observation, whereas the concentration of alcohol in the urine rose. The percentile increase of the concentration of alcohol in the urine

6. Harger, R.: J. Lab. & Clin. Med. **20**:746, 1935.

occurred more rapidly than did the percentile decrease of the concentration in the blood. The rapidity of the urinary alcohol rise was greatest in those animals which had the greatest initial disproportion in concentration of alcohol between the blood and the urine. In dog 4, in which the initial deficit in urinary alcohol was least, the rise in the concentration of alcohol within the first three hours brought it into equilibrium with that in the blood, the ratio of blood alcohol to urinary alcohol being 1 to 1.36.

TABLE 1.—*Passage of Alcohol Into the Urine by Way of the Bladder Mucosa in Living Dogs*

Time, Hr.	Blood Alcohol, Mg. per 100 Cc.	Bladder Alcohol, Mg. per 100 Cc.	Percentile Increase in Concentration of Alcohol in Urine	Ratio: Urine to Blood	Blood Alcohol, Mg. per 100 Cc.	Bladder Alcohol, Mg. per 100 Cc.	Percentile Increase in Concentration of Alcohol in Urine	Ratio: Urine to Blood
	Dog 1				Dog 2			
0	455	69	..	0.15	582	220	..	0.38
½	431	89	29	0.21	579	342	55	0.59
1	414	125	81	0.30	560	418	90	0.75
1½	396	149	116	0.38	550	460	109	0.84
2	375	162	135	0.43	540	488	122	0.90
2½	360	179	159	0.50	536	515	134	0.96
3	353	220	219	0.62	528	542	146	1.03
3½	...	...	...	....	...	...	...	....
4	...	...	...	....	...	...	...	....
	Dog 3				Dog 4			
0	595	350	...	0.59	313	279	...	0.89
½	...	...	...	....	...	...	...	....
1	534	450	29	0.84	307	309	11	1.00
1½	...	...	...	....	...	...	...	....
2	476	500	43	1.05	...	...	...	....
2½	...	...	...	....	...	...	...	....
3	447	527	51	1.18	263	357	28	1.36
3½	...	...	...	....	...	...	...	....
4	436	530	51	1.22	252	344	23	1.37

It is apparent that so far as dogs are concerned equilibrium between blood alcohol and urinary alcohol may be established by direct passage of alcohol through the mucosa of the bladder and that this phenomenon occurs at alcohol levels which may be described as physiologic. If similar diffusion of alcohol between the blood and the urine occurs in similar circumstances through the mucosa of the bladder in man, it would tend to reduce differences in concentration of alcohol between blood and urine which might otherwise be of clinical or medicolegal significance.

Experiment 2. Loss of Alcohol from the Urine by Way of the Mucosa of the Bladder in Living Dogs.—In this experiment 2 dogs (5 and 6) received no alcohol

intravenously, and after the ureters had been ligated and cannulated, between 50 and 100 cc. of urine containing a measured amount of alcohol was injected into the bladder. Samples of blood and urine were withdrawn at intervals in the same manner as in experiment 1. In the case of dog 3 the observations recorded in table 2 represent a continuation of those on this dog recorded in table 1. It may be seen from the last observations on dog 3 recorded in table 1 that the urinary alcohol was higher than the alcohol of the blood and that it had been approximately stationary for an hour.

In table 2 the initial observations on all 3 animals disclose that the urine contained in the isolated bladder had a concentration of alcohol higher than that of a simultaneously collected sample of blood. As in experiment 1, there was no appreciable loss or gain in the total

TABLE 2.—*Loss of Alcohol from the Urine by Way of the Mucosa of the Bladder in Living Dogs*

	Dog 5			Dog 6			Dog 3 (Continued)				
Time, Hours	Blood Alcohol, Mg. per 100 Cc.	Bladder Alcohol, Mg. per 100 Cc.	Percentile Decrease of Concentration of Alcohol in Urine	Blood Alcohol, Mg. per 100 Cc.	Bladder Alcohol, Mg. per 100 Cc.	Percentile Decrease of Concentration of Alcohol in Urine	Blood Alcohol, Mg. per 100 Cc.	Percentile Decrease of Concentration of Alcohol in Blood	Bladder Alcohol, Mg. per 100 Cc.	Percentile Decrease of Concentration of Alcohol in Urine	Ratio: Urine to Blood
0	0	266	....	0	370	....	426	....	530	....	1.22
½	..	228	14%	..	342	7%	...	....	...	....	....
1	..	218	18%	..	330	11%	411	6%	527	1%	1.28
1½	..	209	21%	..	315	15%	...	....	...	....	....
2	..	203	24%	..	298	19%	389	11%	508	4%	1.31
2½	..	193	27%	..	285	23%	...	....	...	....	....
3	..	185	30%	..	272	27%	361	17%	483	7%	1.33
3½	..	180	32%	..	263	29%	...	....	...	....	....
4	Trace	173	35%	Trace	257	31%	300	31%	425	20%	1.42

amount of fluid in the bladder by diffusion or absorption. Although in all 3 animals there was a continuous loss of alcohol from the bladder, the percentile decrease in its concentration did not occur as rapidly as did the increase in experiment 1, when the relative concentrations of alcohol in the blood and the urine were reversed. In other words, it appears that alcohol passes from the blood to the urine by way of the mucosa of the bladder with greater facility than it does from the urine to the blood.

It may be seen, however, that in dog 3 the loss of alcohol from the bladder by diffusion or absorption occurred only slightly less rapidly than did its loss from the blood by excretion and oxidation. The percentile fall in the alcohol concentration of the blood in four hours' time was only slightly greater than that in the alcohol concentration of the urine in the bladder.

It is apparent that so far as the dog is concerned alcohol may be lost from the urine by way of the mucosa of the bladder at what might be described as physiologic levels if its concentration in the urine is disproportionate to that of its concentration in the blood. If a similar loss of alcohol from the urine in the bladder occurs in similar circumstances in man, it would tend to reduce differences in concentration which might otherwise provide information of clinical or medicolegal significance.

Experiment 3. Passage of Alcohol out of and into the Bladder After Death by Way of the Mucosa of the Bladder.—Observations were made on the rate and the circumstances in which the concentration of alcohol in the urine of the bladder may change after death. Three dogs were used in this experiment. In the first the concentration of alcohol in the urine of the bladder was considerably higher

TABLE 3.—*Passage of Alcohol out of and into the Urine by Way of the Mucosa of the Bladder After Death*

	Dog 7				Dog 3 (Continued)				Dog 8			
Time, Hours	Blood Alcohol, Mg. per 100 Cc.	Bladder Alcohol, Mg. per 100 Cc.	Percentile Change in Concentration of Alcohol in Urine	Ratio: Urine to Blood	Blood Alcohol, Mg. per 100 Cc.	Bladder Alcohol, Mg. per 100 Cc.	Percentile Change in Concentration of Alcohol in Urine	Ratio: Urine to Blood	Blood Alcohol, Mg. per 100 Cc.	Bladder Alcohol, Mg. per 100 Cc.	Percentile Change in Concentration of Alcohol in Urine	Ratio: Urine to Blood
0	72	405	....	5.62	300	425	...	1.42	435	198	....	0.46
4	..	340	−15	....	...	433	+2	....	...	256	+29	....
8	..	314	−25	....	...	420	−1	....	...	263	+33	....
20	..	260	−35	....	...	415	−2	....	...	311	+57	....

than that in the blood at the time of death. In the second the concentration of alcohol in the urine of the bladder was in approximate equilibrium with that in the blood at the time of death. In the third the concentration of alcohol in the urine of the bladder was considerably lower than that in the blood at the time of death. Samples of blood and urine were taken at intervals throughout the postmortem period. The bodies of all 3 animals were kept at room temperature (about 22 C.).

The results observed in these 3 animals are recorded in table 3.

It will be noted that in animal 7, in which at the time of death the concentration of alcohol in the urine of the bladder was considerably higher than the concentration in the blood, there was a progressive loss of alcohol from the bladder and that at the end of twenty hours the concentration had been reduced from 405 to 260 mg. per hundred cubic centimeters. That this reduction was due to diffusion incident to the establishment of equilibrium between the urine in the bladder and its environment may be seen by comparing the analytic results recorded for dog 7 with those for dogs 3 and 8. In the case of dog 3 there was

relatively little difference between the alcohol concentration of the blood and that of the urine at the time of death. The ratio of blood alcohol to urinary alcohol at the time of death was 1 to 1.42, which was probably not far from equilibrium. No significant change in the concentration of alcohol in the urine occurred during the postmortem interval of twenty hours. In dog 8 the concentration of alcohol in the blood at the time of death was considerably higher than that of alcohol in the urine of the bladder. It may be seen that there was a progressive increase in the concentration of alcohol in the urine of the bladder throughout the postmortem interval from an initial level of 198 mg. per hundred cubic centimeters to a terminal level of 311 mg. per hundred cubic centimeters.

In none of these animals was there any significant gain or loss in the total amount of fluid in the bladder.

COMMENT

In each of a series of dogs the bladder was isolated from the kidneys by ligation and section of the ureters. Subsequent to this procedure, arbitrary initial differences were established between the concentration of alcohol in the blood and that in the urine contained in the isolated bladder. All concentrations were within the normal range in the sense that none of them was higher than might result from drinking an alcoholic beverage. The initial disparity between the concentration of alcohol in the blood and that in the urine of the bladder was greater in some animals than would be likely to occur in nonexperimental conditions.

It was found that the concentration of alcohol in the blood and that in the urine of the isolated bladder tended to approach equilibrium as a result of passage of alcohol into or out of the bladder by way of the mucosa. Equilibrium between the concentration of alcohol in the blood and that in the urine in the isolated bladder was approached more rapidly when the original disproportion was in favor of the blood than it was when the original disproportion was in favor of the urine. It appeared that diffusion of alcohol into the bladder in the presence of a relative deficit in the urinary alcohol was a more potent factor in establishing equilibrium than was diffusion of alcohol into the blood. The rate at which alcohol passed through the mucosa of the bladder was modified in accordance with the magnitude of the initial difference between the alcohol contents of the two fluids. Other things being equal, the greater the original difference in concentration of alcohol the more rapid was the movement of alcohol through the mucosa of the

bladder. The concentration ratio between the blood and the urine of the bladder which was approached by this diffusion appeared to be similar to that which is observed normally.

It may be inferred from these observations that during the period of rising concentration of alcohol in the blood such as occurs after the ingestion of an alcoholic beverage two processes may contribute to the rising concentration of alcohol in the urine of the bladder. One is the excretion of alcohol by the kidney, and the other is the diffusion of alcohol directly through the mucosa of the bladder. Whereas the former probably occurs as long as alcohol is present in the circulating blood, it appears that the latter occurs only so long as the disequilibrium in concentration between the blood and the urine of the bladder is in favor of the former.

In animals in which the concentration of alcohol in the urine of the bladder was so much higher than the concentration in the blood that a disequilibrium was established between the two solutions, alcohol passed out of the bladder by way of the mucosa. The loss of alcohol from the urine by way of the mucosa tended to bring the alcohol content of the urine closer to a state of equilibrium with that of the blood.

Diffusion of alcohol into or out of the urine by way of the mucosa of the bladder is not an exclusively vital phenomenon. In dogs it progressed after death although at a relatively slower rate than it did in living animals. Disproportionate differences in concentration of alcohol between the blood and the urine of the bladder at the time of death were reduced by the passage of alcohol into or out of the urine by way of the mucosa of the bladder.

CONCLUSIONS

The urine in the bladder does not represent an unaltered pool of ureteral urine so far as the concentration of the alcohol in it is concerned.

Alcohol may pass into the urine by way of the mucosa of the bladder if the concentration of alcohol in the bladder content is disproportionately low in relation to that in the blood, and out of the urine by the same route if the concentration of alcohol is disproportionately high.

The passage of alcohol through the mucosa of the bladder may occur in either direction after death, as well as in life, and the direction of the diffusion is determined by the relative concentrations of alcohol in the blood and the urine at the time of death.

It is not necessary that either the absolute or the relative concentrations of alcohol in the urine and the blood be different from those which occur normally in order for an interchange of alcohol between the urine in the bladder and the blood to occur.

In both living and dead animals the passage of alcohol through the bladder's mucosa tends to bring the concentrations of the alcohol in the blood and the urine closer to, rather than farther from, a state of equilibrium.

The fact that alcohol may pass through the mucosa of the bladder in either direction tends to enhance rather than to detract from the clinical and medicolegal significance of any disproportion which may exist between the concentrations of alcohol in simultaneously collected samples of blood and bladder urine.

Notes and News

Appointments.—René Dubos, member of the Rockefeller Institute for Medical Research, has been appointed George Fabyan professor of comparative pathology and professor of tropical medicine in Harvard Medical School, Boston, succeeding Ernest E. Tyzzer, who becomes emeritus.

Robert F. Loeb, professor of medicine, Columbia University (College of Physicians and Surgeons), has been named Lambert professor of medicine in that institution.

Béla Halpert, assistant professor of pathology in the Louisiana State University, has been appointed director of the laboratories of the hospitals of the University of Oklahoma.

George W. Thorn, associate professor of medicine in the Johns Hopkins University, has been appointed Hersey professor of the theory and practice of physic in Harvard Medical School and physician-in-chief of the Peter Bent Brigham Hospital, succeeding Soma Weiss, who died Jan. 31, 1942.

Awards.—The Distinguished Service Medal of the American Medical Association for 1942 was awarded at the Atlantic City Session of the Association to Dr. Ludvig Hektoen, who has been chief editor of the ARCHIVES OF PATHOLOGY since its inception. This award, the greatest token of distinction in science offered by the American Medical Association, was tendered to Dr. Hektoen as a recognition of the tremendous contribution which he has made to the science of medicine. In 1929, the Norwegian government gave Dr. Hektoen its most distinguished recognition, the Order of St. Olaf, and in 1941, as a native of Wisconsin, he was awarded the Distinguished Service Award of the State Medical Society of Wisconsin. [M. F.]

The William Osler Medal of the American Association of the History of Medicine has been awarded to John T. Barrett of the Boston University School of Medicine.

The Kober Medal of the Association of American Physicians has been awarded to Donald D. Van Slyke, member of the Rockefeller Institute for Medical Research, in recognition of "his contributions to the treatment of diabetes and Bright's disease as well as for his work on the oxygen treatment of pneumonia."

The 1941 Strittmatter Medal of the Philadelphia County Medical Society has been awarded to Joseph McFarland, emeritus professor of pathology in the University of Pennsylvania.

The Copley Medal of the Royal Society, London, has been awarded to Sir Thomas Lewis for his study of clinical circulatory problems by experimental methods.

Histopathologic Research Institute.—According to *Science,* the Institución Cultural Española in Buenos Aires has established a laboratory for histopathologic research in memory of S. Ramón y Cajal to be directed by Pío del Río Hortega, of Buenos Aires, with a staff of eight investigators. The laboratory will conduct investigations on the histology of the nervous system, employing the technic of Cajal and his school.

Society News.—The Biological Photographic Association will hold its twelfth annual convention in New York, Sept. 10, 11 and 12, 1942.

Book Reviews

Rabies. Leslie T. Webster, M.D., The Rockefeller Institute for Medical Research, New York. Pp. 168. Price $1.75. New York: The Macmillan Company, 1942.

The first part of this little book deals with the diagnosis of rabies. The distinctive features of the disease in animals, particularly the dog, of course, and in man, and the manner of its causation are considered. Special emphasis is put on the fact, and on the consequences thereof, that there are no distinctive tests for rabies that may be used during the life of the victim. The postmortem mouse inoculation test devised by the author, which marks a great advance in the means of studying the problems of rabies, is now the "ultimate court of appeal in all doubtful cases" and is used as a routine method of diagnosis of rabies in some twenty states.

The second part of the book is devoted to the prevention of rabies. The chapter on the history and the epidemiology of the disease contains instructive tables and maps of the distribution of the disease in animals and in man in the United States. There are chapters on measures for preventing rabies before as well as after exposure and on methods of evaluating antirabic vaccines, among which the mouse test is of great value. There are appendixes on the disposal and the quarantine of dogs, on rabies antibodies and on the potency of antirabic vaccines. The conclusions from the discussions on vaccination against rabies merit quotation. "Persons exposed to rabies should be given vaccine treatment with confidence that then there is small likelihood of development of the disease," but the investigations of the efficacy of the treatment should be continued from all angles. And "rabies vaccines designed for public consumption should be required to possess a definite immunizing capacity, as determined by the mouse potency test. The amount of immunizing potency required remains to be determined. Second, persons exposed to rabies should receive antirabies treatment only with vaccines proved potent according to the mouse test. Third, whereas prophylactic immunization against rabies is a definite possibility which is engaging the attention of groups of workers in several laboratories, no vaccine has yet been developed with sufficient qualifications to justify compulsory vaccination of dog or other animal populations. Rather, attention should be paid to the progress of experiments in this field dispassionately and critically and with confidence that if a satisfactory vaccine is developed, it will be adopted with enthusiasm." These are well founded statements of great importance. Rabies is a community problem requiring continued research. Dr. Webster's book will be of interest and value to all who are concerned with rabies whether as public health officials, physicians, veterinarians, investigators or dog owners. It fills well the urgent need for a competent book on the diagnosis and the control of rabies based on the present state of knowledge of the disease.

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